GENETICS

A periodical record of investigations bearing on heredity and variation

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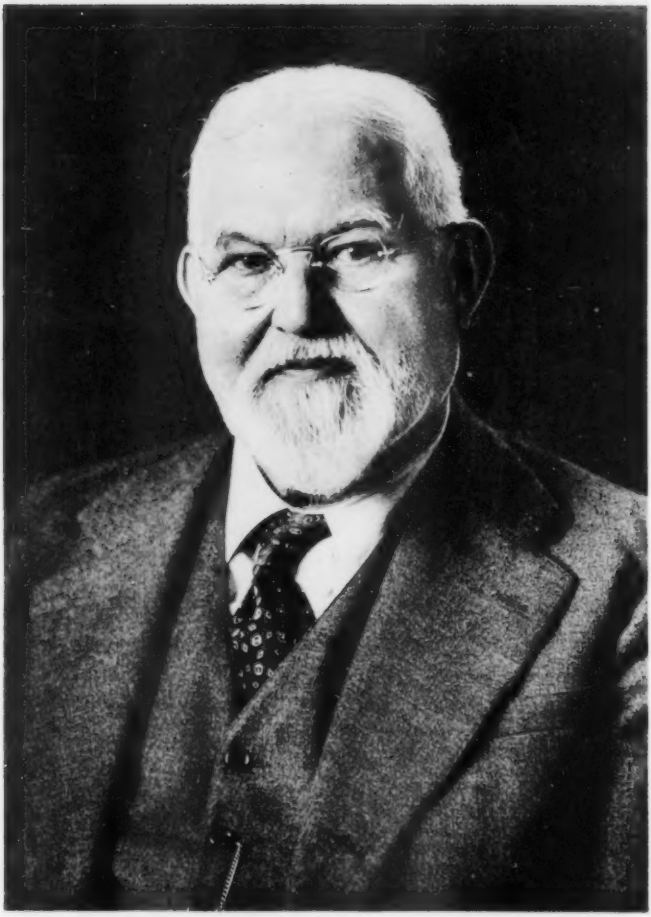

Sincerely yours
Geo. H. Shull

GEORGE HARRISON SHULL

THE life of George Harrison Shull is the familiar but always-inspiring story of a young man, reared under modest circumstances, who, through courage, hard work and devotion to ideals, overcame all odds and obstacles to create a successful and distinguished career in his chosen field.

Shull was born on a farm in Clark County, Ohio on April 15, 1874, the fifth of eight children of Harrison Shull and his wife, Catherine Ryman. The father, a farmer, was also a faithful member of the Old German Baptist Church and at times an unpaid lay minister to the congregation. The mother, always interested in serious reading, became, after her children were reared, an accomplished amateur horticulturist with a first-hand knowledge of plants.

The family moved repeatedly, occupying eight successive rented farms while young Shull was still a barefoot boy. This circumstance, combined with the need for his services on the farm in fall and spring, kept his formal schooling to a minimum, and when he entered college he had spent only 46½ months in the classroom. Formal schooling was, however, supplemented by home study in which the entire family participated. The effectiveness of this self-education (as well as the genetic excellence of the Shull stock) is attested by the fact that all of the seven living children later became teachers in the public schools and that four are listed in "Who's Who."

Shull's interest in the study of plants began at the age of ten when he joined his eldest brother, Samuel Peter, then eighteen, in the study of botany. With a volume of Wood's "Object Lessons in Botany" under his arm, young Shull roamed the woods and countryside searching for new wild flowers to identify by the simple keys which the book contained. Later, when George was sixteen, another elder brother, John William, suggested that he "learn everything that was known about plants and then go on to make new discoveries." From that day on Shull determined to make the study of plants his life work and despite numerous difficulties never lost sight of this ultimate goal.

From 1892 to 1901 Shull divided his time between teaching in the public schools of Ohio and attending Antioch College where he made the remarkable average grade of 97 in the four-year course. He received his degree in June 1901 at the age of 27.

In September of 1901 Shull entered the University of Chicago as a graduate student. Within a few months he was appointed, on the basis of a Civil Service examination taken earlier, as Botanical Assistant in the U. S. National Herbarium. Arranging to have his work in the Herbarium count as residence credit in the University, Shull proceeded to Washington. At the end of three months of service in the Herbarium he was transferred to the U. S. Bureau of Plant Industry with the title of Botanical Expert and the task of making a botanical study in Chesapeake Bay and Currituck Sound of the food plants of wild ducks, whose numbers had in recent years diminished alarmingly. These studies, pursued through two summers, were to have become the subject of his Ph.D. thesis at the University but other developments intervened.

In his last year at Antioch Shull had made a statistical study of variation in the floral parts of four species of wild asters which he presented to the college authorities

in lieu of a commencement oration. At Chicago he became further interested in biometrical studies through his association with Dr. Charles B. Davenport, one of the distinguished pioneers in this new field. When Davenport, in 1904, was appointed Director of the newly established laboratory of the Carnegie Institution at Cold Spring Harbor, Long Island, Shull applied for and received an appointment on the staff, being placed in charge of investigations in variation, modification and heredity in plants. Substituting as his Ph.D. thesis a nearly completed paper entitled "Place Constants for *Aster prenanthoides*" for the previously planned "Ecological Survey of Chesapeake Bay," he proceeded to Cold Spring Harbor. There on May 2, 1904 he began the life work which brought him world-wide recognition as a leader in the new, still nameless science which two years later was to be christened "genetics."

As subjects for experimental study Shull chose the evening primrose and the shepherd's purse, and his work on these species continued without interruption from 1904 to 1942 when he retired from his Professorship at Princeton University to which he been appointed in 1915. During this period he grew more than a million pedigreed specimens of shepherd's purse and three quarters of a million specimens of evening primrose. He published fifteen papers on the former and twenty-two on the latter. Other genera which attracted his interest and became subjects of his studies and experiments were Zea, Phaseolus, Lychnis, Fraxinus, Digitalis, Helianthus, Lycopersicum, Papaver and Solanum. His list of published papers comprises 167 titles and covers a period of more than 60 years, the first paper appearing in 1891, the last in 1953.

Shull had a flair for inventing and adapting technical terms. Among those of his initiation which have come into common usage are "duplicate genes," "sib," "geneticist" and "heterosis."

Although Shull's investigations in theoretical genetics have been numerous, diverse and widely recognized, they have been completely overshadowed by a single, truly monumental contribution which he made in the field of applied genetics: the development of hybrid corn.

Hybrid corn has been termed "the most important development in applied biology of this century." Like most advances in the application of science, hybrid corn is the work of many men and the product of many minds. To call any one person the "father" or "inventor" of hybrid corn is to overlook the significant fact that spectacular achievements in applied science usually rest upon a solid but sometimes inconspicuous foundation of theoretical science. Shull could do what he did because he knew and understood the earlier work of Mendel, Darwin, Galton and Johannsen. And he was capable of exploiting this knowledge in the improvement of the maize plant because of his boyhood experience in practical farming. This unique combination of circumstances was indeed a fortunate one both for Shull and for American agriculture.

Shull had no thought of improving the maize plant when in 1905 he began his studies on it. Instead he started with the objective of analyzing the inheritance of quantitative or "blending" characters and he chose the number of rows of kernels in ears of maize as an inherited and easily measured character suitable for study. Through self-pollination he developed a number of inbred lines differing in average

kernel-row number. These lines, as a consequence of inbreeding, declined in vigor and productivness and at the same time each became quite uniform. Shull concluded quite correctly that he had isolated pure lines of maize similar to those which Johannsen had recently described in beans.

As a first step in studying the inheritance of kernel-row number, Shull crossed these pure lines. The results were surprising and highly significant. The hybrids between the pure lines were, like their inbred parents, quite uniform, but, unlike their parents, they were vigorous and productive. Some were definitely superior to the original open-pollinated variety from which they had been derived.

From his experiments Shull recognized, and was the first to do so, "the reciprocal relation between the deterioration due to inbreeding and the increased vigor due to crossing, as the two aspects of a single phenomenon." More important still, he saw at once that inbreeding followed by crossing offered an entirely new method of improving yield in corn. In two papers published in 1908 and 1909 he reported his results and outlined a method of corn breeding based upon his discoveries. He pointed out the important advantages which his proposed program offered to agricultural practice: greater yields; greater uniformity; sharper specialization to fit different regions, climates and soils; the production of strains of any desired chemical content or other qualities.

To read Shull's two papers in the light of the present worldwide use of hybrid corn is a revelation. That Shull should have been able, from the limited experiments then completed and from unreplicated yield tests, not only to draw valid conclusions regarding the effects of inbreeding and crossbreeding, but also to design a new method of corn breeding based upon the exploitation of heterosis, was either inspired intuition or a stroke of genius. Certainly it is one of the most remarkable achievements of our time in the field of applied biology. Shull's idea of producing and maintaining otherwise useless inbred lines of maize solely for the purpose of utilizing the increased vigor and uniformity resulting from their hybridization was revolutionary as a method of corn breeding. It is still the basic principle which underlies almost the entire hybrid corn enterprise.

But Shull's proposed method of hybrid corn production, involving the crossing of two inbred strains, proved to have one serious shortcoming—the cost of seed production was prohibitive. When this was overcome by Jones' invention of the double cross in 1918, hybrid corn production became immediately practicable and Shull lived to see his magnificent design transformed into reality on a grand scale. By 1933 hybrid corn was being grown on a commercial acreage large enough to justify the collection of annual statistics. During the forties the increased production resulting from the use of hybrid corn contributed not only to this country's war effort but also to the rehabilitation of Europe after the war. Today almost 90 percent of the U. S. corn acreage is planted to hybrid corn and it has also become important in France, Spain, Italy and the countries of Latin America. Shull's part in this tremendous development has been widely recognized and he received numerous citations and awards, including a gold medal from the DeKalb Agricultural Association in 1940, the John Scott Award and medal presented by the City of Philadelphia in 1945 and the Marcellus Hartley Medal, commonly called the "Public Welfare Medal," by the National

Academy of Sciences in 1948. Shull was also honored by election to numerous scholarly societies in the United States and abroad and by honorary doctorates conferred by Lawrence College and Iowa State College.

Shull's personal life, like his professional career, was rich in interesting and rewarding experiences. Beginning with his service as a young teacher in Ohio, he maintained a life-long interest in public schools. He served for sixteen years as a member of the Princeton Borough Board of Education, the last six years as its president. During his membership on the Board, three modern school buildings were built and the Princeton Borough public school system gained recognition throughout the country for its excellence. Since his retirement in 1942 he gave much time and effort to the preservation of Island Beach on the New Jersey Shore as a wild-life sanctuary. His wife, Mary Julia Nicholl, whom he married in 1909, is the mother of four sons and two daughters who have presented Dr. and Mrs. Shull with 23 grandchildren.

It seems appropriate that a biography of Shull appearing in GENETICS should end with a review of the part which he played in its history. Shortly after accepting the professorship at Princeton University, Shull, recognizing the need of a journal to publish technical contributions in the rapidly expanding field of genetics, arranged with the Princeton University Press to undertake the publication of such a journal on condition that at least 250 advance subscriptions be secured. He organized an editorial board of nine distinguished American geneticists, all of whom are either still serving or did serve on the board until their deaths. He remained managing editor for the first ten years during which the journal became financially established, and he raised the special "Galton and Mendel Memorial Fund," whose proceeds are used to finance illustrations and tabular matter in GENETICS.

His interest in GENETICS continued unabated to the end and he was in frequent correspondence with the editors on matters of policy and details of publication. To recognize his many years of devoted and distinguished service to the journal which he founded, its present editors, making an exception to long-established policy, had planned to publish, while he still lived, his portrait and a biographical sketch. Dr. Shull's death on September 29, 1954 denied him this final tribute; but it was a source of satisfaction to him to know that Volume 40 of the journal which he had founded, to which he had given so much of his life and which, in turn, had become so much a part of his, would begin with this biography.

PAUL C. MANGELSDORF

POSITION EFFECTS AT THE BROWN LOCUS IN DROSOPHILA MELANOGASTER[1]*

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THE term "position effect" refers to the differential effects in the action of a gene which are dependent on its position on the chromosomes. The method used here for the investigation of this problem was to select the brown eye color locus (*bw*, 2-104.5) in *Drosophila melanogaster* and study the range of effects which could be induced in two alleles. One of these appears to be a normal wild type allele. The other is a recessive of unknown, but presumably spontaneous origin. This recessive allele and others like it cannot be shown to be producing any gene product and it may be assumed to be amorphic.

By choosing an eye color, a number of simplifications in the interpretation of the data might be expected. Also, brown is in a relatively well studied region of the salivary gland chromosomes, as BRIDGES (1937) investigated this area, and banding near the brown locus is easily identified. Only one other eye color, purpleoid (*pd*, 2-106.4), is near brown. It appears that alleles of purpleoid have not been found in this experiment. Brown has been shown to be sensitive to chromosome rearrangements, carrying either a wild or a recessive allele (see GLASS 1933).

THE INDUCTION OF EYE COLOR MUTANTS

Methods

WRIGHT (1932) noted that the combination of brown and scarlet (*st*, 3-44.0) shows no eye pigment. For this reason, scarlet was used throughout these experiments since it magnified the small color difference between wild and brown to the great difference between scarlet and white.

An isogenic stock was established from crosses of a *ClB*/+; *Cy-RNS*/+; *Payne Dfd ca*/+ female to a *bw*; *st* male. A daughter carrying all three of the female's dominants was crossed to a phenotypically wild type brother. Daughters of this cross which carried all three dominants were crossed to their own father and in one case some *bw*; *st* offspring were found. Two flies from this culture were mated as founders of a *bw*; *st* stock and another *bw*; *st* male was mated to a +/*bw*; *st* sister. Thereafter, the *bw*; *st* stock was continued by single pair matings of a brother and sister, and the +/*bw*; *st* stock was continued by breeding a male from the same generation of the *bw*; *st* stock to a +/*bw*; *st* female. The crossing technique used should make the first and third chromosomes isogenic in both lines and the second chromosome isogenic in the *bw*; *st* stock, except perhaps for rare mutations or crossovers. The inbreeding practice would tend to give isogenicity if not attained through

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[1] This paper is largely from a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, Department of Zoology, University of Chicago.

the crossings, and would give the greatest likelihood of the second chromosome becoming isogenic in the $+/bw$; *st* line except in the immediate vicinity of the brown locus. The first irradiation was performed after ten generations of such inbreeding, the last after forty-eight.

The preparation of stocks for an irradiation was begun by mating a pair of $+/bw$; *st* flies. By utilizing progeny testing, one or more pairs of bw^+ homozygotes were found in a short time. At the same time, one or more pairs of *bw*; *st* sibs of the bw^+ homozygotes were chosen. From these sibling bw^+ and *bw* homozygous pairs, a number of pairs of offspring were mated to give the larger numbers of the two types of offspring needed for irradiation. Thus, the flies in the irradiated generation were all the F_3 of a single brother-sister pair.

The F_2 pairs were transferred at frequent intervals and their progeny emerged over a relatively long period of time. Adults of the F_3 were collected frequently to insure the virginity of both sexes. The males were irradiated at ages varying from newly emerged to four days after emergence and mated to females within a similar age span. The dosage used was 4000 r of X-rays at 250 kilovolts. As a precaution against irregularities due to deviations in the dosage administered, an attempt was made to have approximately equal numbers of matings in each category of the experiment in each irradiation. Both eyes of all F_1 flies were examined for color changes. Mutants were numbered in the order of their discovery.

Both bw^+; *st* and *bw*; *st* males were mated to females of each genotype. This gave four categories ("series") of progeny, as follows:

Series	Male Genotype (Irradiated)	Female Genotype (Not Irradiated)
A	bw^+; *st*	bw^+; *st*
B	*bw*; *st*	bw^+; *st*
C	bw^+; *st*	*bw*; *st*
D	*bw*; *st*	*bw*; *st*

The results of this experiment made it advisable to run a special experiment limited to series *B* and *C*. It was necessary to mark the second chromosomes so that they could be identified as maternal or paternal. Plexus (*px*, 2-100.5) and speck (*sp*, 2-107.0) were chosen to mark the female (non-irradiated) chromosomes and stocks were made up which were *px* bw^+ *sp*; *st* and *px bw sp*; *st*. The bw^+, *bw* and *st* alleles in these stocks were derived from those in the inbred lines. These new stocks and a bw^+; *st* stock were maintained in mass cultures. The *bw*; *st* flies were taken from the inbred line. The irradiation dosage and accompanying techniques were the same as used in the preceding experiment. The experimental series have been designated B' and C' and the crosses are comparable with the *B* and *C* series.

The Rate of Induction of Eye Color Mutants

The *A*, *B*, B', *C* and C' series gave seventy-eight eye color mutants of which fifty-five were fertile. Three of these cannot be considered, as their offspring were not followed far enough to gain information about the irradiated second chromosome. Seven mutants appear to be due to changes at loci other than brown. Of the forty-five remaining mutants, ten in the *C* series were phenotypically white and were shown by test to be due to mutation at the brown locus. They showed no difference be-

TABLE 1

The distribution of irradiation induced eye color mutants

Series	Number of offspring examined	Eye color mutants	Probably brown locus	Known brown position effects	Mutant fly phenotypically brown
A	34,682	11	7	4	0
B	38,621	12	10	6	0
C	37,911	29	27	8	12 (+3)
D	22,835	0	0	0	0
B′	11,832	3	2	0	0
C′	23,474	23	19	6	5

tween the irradiated chromosome and its homologue. These stocks had to be discarded. Two stocks have been derived from what appear to have been *bw* somatic mosaics which transmitted the wild allele to their progeny. The other stocks consist of three iso-alleles; two recessive non-variegated intermediate alleles, four amorphs, one deficiency and twenty-three variegated position effects at the brown locus. Under the conditions of this experiment, detectable mutants are more frequent at the brown locus than at all other eye color loci combined. Table 1 shows the number of mutants found in each series of the experiment, the number of mutants which were probably due to any change at the brown locus and those known to be due to brown locus position effects. The last column gives those cases in which the original mutant was white (*bw*; *st*). The separate group of three in the *C* series are stocks which were white in the mutant fly but whose descendants have been shown to carry position effects rather than point mutations.

From the lack of mutants in the *D* series it appears that the rate of formation of mutants which produce pigment in *bw*; *st* flies is negligible, or zero. For the total number of mutants, the *C* plus *C′* series differ from the *A* and from the *B* plus *B′* series by highly significant values. This is also true if only those mutants which are likely to have been at the brown locus are considered. However, these differences are accentuated by the occurrence of point mutations. Such alleles would not have been detected in the *A*, *B* and *B′* series. If correction is made for these alleles, restricting the calculations to variegated *bw* position effects, there probably were seven among 34,682 flies in the *A* series, twelve among 50,453 in the *B* plus *B′* series and twenty-three among 61,385 in the *C* plus *C′* series. These values do not differ from each other significantly, the comparable series, *B* plus *B′* and *C* plus *C′*, have a *P* value between .3 and .2. Similarly, there is no significant difference in the rates of formation of position effects if only the more adequately studied cases are considered.

Point Mutations at the Brown Locus

The symbols used in this paper are adapted from those of STERN and HEIDENTHAL (1944), who designate a rearranged chromosome by the letter "*R*". The symbol in parentheses shows whether the irradiated allele was wild type or recessive. The number of the stock has been written as a superscript.

The mutant ancestor of stock $(+)^{77}$ was dark brown in one eye and normal in the

other; that of stock $(+)^{70}$ had one eye which was normal except for one small spot while the other eye had only small pigmented areas on a white background. Both resembled *bw* position effects but they transmitted normal wild type alleles which show no changes in their salivary gland chromosomes. They are presumed to have had unchanged wild type alleles in the germ cells, whatever may have happened in some of their somatic cells. The mutant ancestor of stock $(+)^{21}$ also had one eye variegated and the other eye normal. The salivary gland chromosomes are normal. Nevertheless, a mutation seems to have persisted in this case since the amount of pigment appears to be reduced in combinations with position effects (see table 3). It is assumed to be an iso-allele. All three of the above mutants occurred in the *C* or *C'* series.

All four of the clearly intermediate alleles arose in the *C* and *C'* series. The failure to find intermediate alleles in the *B* and *B'* series or among the many other heterozygous flies which have been observed, makes it probable that they were irradiation induced. Three of these mutants, $(+)^{59}$, $(+)^{75}$ and $(+)^{81}$ show no chromosomal abnormality, but $R^{24}(+)$ is limited to males, and it appears that the region from 45A to 51E, which is on the second chromosome well to the left of the brown locus, has been inserted into the Y chromosome. The brown region (in 59) appears normal. Linkage of the second chromosome with sex is not complete. Males failing to receive the $R^{24}(+)$ chromosome (but having its normal homologue) are sterile. Females with the broken second chromosome have not been recovered. The mutant is associated with a dominant roughness of the eye facets which is almost completely penetrant, and which can be used to identify the mutant in crosses. It is assumed that the eye color is due to a point mutation which occurred independently of the associated translocation. The $(+)^{59}/(+)^{59}$ homozygotes are not clearly distinguishable from wild type, and so this mutant must be regarded as an iso-allele. $R^{24}(+)$ is very much like $(+)^{59}$ in crosses and $R^{24}(+)/(+)^{75}$ is wild type whereas $(+)^{75}/(+)^{75}$ is not. Therefore, it appears that $R^{24}(+)$ should be classified as an iso-allele too. $(+)^{75}$ and $(+)^{81}$ are clearly recessive to wild type. All four mutants will be discussed as intermediate alleles.

$R^{68}(+)$, $(+)^{69}$ and $(+)^{72}$ have white eyes in combination with *bw*. They occurred in the *C'* series, which was designed to pick up and identify such mutants. $R^{68}(+)$ is associated with a complex rearrangement which is no closer to the *bw* locus than 58F. Homozygotes occur, but cannot be maintained as a stock. It is presumed that the change at the brown locus occurred at the same time as an unrelated rearrangement. The salivary gland chromosomes are normal in $(+)^{69}$ and $(+)^{72}$.

THE CYTOLOGY OF THE BROWN LOCUS

The right end of the second chromosome, which contains the brown locus, has been drawn by BRIDGES (1935, 1937) and BRIDGES and BRIDGES (1939). The drawing by BRIDGES (1937) represents the end of the chromosome as it is usually seen. On rare occasion the chromosome may be stretched so that most of the bands drawn by BRIDGES and BRIDGES (1939) can be seen.

A few variegated brown mutants have been shown to be due to rearrangements in the 59D or 59E region of the salivary gland chromosomes (BRIDGES and BREHME 1944).

TABLE 2

The cytology of brown locus position effects

Series	Position effect	Break in region 59	Chromocenter	Presumed order
B	$R^{3}(bw)$	D8/9	40F	60-59D9/40F-51F/59D8-57E/55E-57E/51F-55E/40F-21.
B	$R^{1}(bw)$	E1·2/3	80–81	60-59E3/80-61; 100-81/59E2-21.
A	$R^{12}(+)$	near D9·10	80C	60-59D/80C-100; 61-80C/59D-21.
C	$R^{14}(+)$	E1·2/3	80	60-59E3/80-100; 61-80/59E2-21.
A	$R^{15}(+)$	near D9·10	80C	60-59D/80C-100; 61·80C/59D-21.
(A)	$R^{18}(+)$	E4/F1	40F–41A	60–59F1/41A-59E4/40F-21.
C	$R^{20}(+)$	D5/6	40D	60-59D6/40D-59D5/40D-21.
B	$R^{25}(bw)$	near D6	near 101E	60-59D/101E-101A; 102-101E/59D-21.
B	$R^{27}(bw)$	D11/E1	Y	60-59E1/Y; Y/59D11-21.
C	$R^{32}(+)$	near D8	41A	60-59D/41A-59D/41A-21.
A	$R^{33}(+)$	near D10	near 41A	60-59E/41-59D/41-21.
A	$R^{35}(+)$	D11/E1	40F-41A	60-59E1/41A-59D11/40F-21.
C	$R^{40}(+)$	D1 to E1·2	—	60-59E3/59C5-21.
B	$R^{45}(bw)$	E3/4	40F-41A	60-59E4/41A-59E3/40F-21.
C	$R^{47}(+)$	D11/E1·2	40–41	60-59E2/41-59D11/40-21.
C	$R^{50}(+)$	D1·2/3·4	Unident.	?
C	$R^{55}(+)$	D3·4/5	40F-41A	60-59D5/40F-24E1/42E-59D4/41A-42E/24D-21.
C′	$R^{56}(+)$	near D11	40F-41A	60-59E/41A-59D/40F-21.
C′	$R^{57}(+)$	D5/6	Y	60-59D6/Y; Y/59D5-21.
C′	$R^{58}(+)$	near D9	101C	60-59D/101C-102; ?/65-100; 61-65/59D-21. 101A-C lost?
C′	$R^{67}(+)$	D1·2/3·4	40F-41A	60-59D3/41A-59D2/40F-21.
C′	$R^{73}(+)$	E4/F1	40F-41A	60-59F1/41A-59E4/40F-21.
C′	$R^{79}(+)$	F1·2/3	40F-41A	60-59F3/41A-59F2/40F-21.

The *bw* position effects which have been analysed here have contained one break in 59D, 59E or 59F. Table 2 lists, for each of the position effects studied, the series in which it was found, the position of the break in region 59, the chromocentral region attached to it and the presumed order of the reconstituted chromosome(s). In addition to the position effects found in these experiments, one additional position effect $R^{18}(+)$, was found in an ancillary experiment under conditions identical with those of series A. All of these position effects are of the variegated type except the deficiency, $R^{40}(+)$, which is dominant for a slight dilution of color but never shows any variegation. The location of breaks in region 59 is probably accurate with respect to bands 59D1·2, D3·4, E1·2 and F1·2. It is doubtful if any of the breaks involving other bands in this region are in error by more than two bands. Breaks in other regions have not been identified as accurately as those in region 59.

The position of the breaks in region 59 gives few clues to the exact position of the brown locus. The breaks are distributed almost randomly throughout the space between 59D1·2 and 59F3. It is most likely that the brown locus is in the region between D8 and E1·2, but the information for several other loci which has been given by DEMEREC (1941) indicates clearly that the brown locus might be anywhere within this region. Corroborative evidence is derived from the deficiency,

$R^{40}(+)$, which also suggests that the locus is within the regions 59D or 59E. If it is assumed that heterochromatin is responsible for variegation, it may be noticed that in $R^{8}(bw)$ and $R^{58}(+)$ the region to the right of about 59D8 is now adjacent to heterochromatin but not the region to the left of this. Therefore, the brown locus is probably to the right of 59D8, although to the left of 59F1.

The chromocentral region which is involved with the brown locus in these breaks is the center of chromosome 2 in thirteen cases, the center of chromosome 3 in four cases, chromosome 4 in two cases and the Y chromosome in two cases. The X chromosome has not been identified in any case, and a survey of the position effects listed by BRIDGES and BREHME (1944) and more recent publications has failed to show any other brown locus position effects which are associated with the X chromosome. The numbers are small and this might be due to an accident of sampling. On the other hand, the large number of inversions as compared with the smaller number of translocations with the presumably comparable chromocenter of chromosome 3 is less likely to be due to a random occurrence. There is a possibility that the two types of breaks are formed in different numbers or that there is a greater likelihood for the translocations to be sterile or inviable.

THE ACTION AND INTERACTION OF BROWN POSITION EFFECTS

Methods

After a number of position effects had been collected, they were crossed to the various alleles of brown and to the other brown position effects. The appropriate progeny were examined and graded for eye color. Where homozygous flies could not be obtained for crossing, the stock was made heterozygous for a completely penetrant dominant wing mutant which has been irradiation induced in this experiment. It is on the second chromosome far to the left of brown, but only rarely crosses over with most brown position effects and is completely balanced by some.

An arbitrary color scale ranging from zero, or white, to six, or scarlet, was used. The intermediate steps were artificially defined for ease in grading. Grade one consisted of eyes in which the pigmentless area constituted the greater part of the surface. Eyes of light pigmentation with few dark spots or with pigmentless areas covering less than half of the surface were in grade two. Grade three eyes were slightly darker but clearly showed a less intense pigmentation than scarlet, and usually were of a browner color. Grade four included all eyes of strong pigmentation which could be distinguished by color, or slight differences in color and intensity, from scarlet. Grade five eyes appeared to be scarlet unless viewed by the shadow technique discussed below. Under these special conditions eyes would be classified as grade five because of deviation from scarlet in as small an area as a single facet. Many crosses show little variation on this scale and almost all genotypes fall into a unimodal curve. Because of the strong sexual dimorphism in many stocks, the sexes were counted separately, and as some mutants show little correlation between the two eyes of the same fly, only the right eye has been used in calculating the figures given. These values are averages of twenty-five individuals wherever possible. Averages based on fewer individuals have been noted.

The unimodal distribution of the grade of eye color is usually accompanied by a narrow range, so that the standard errors of the values in table 3 are very small.

Where twenty-five individuals have been counted, the maximum standard errors are about ±0.25 and usually are less than ±0.10. Values precise to a unit (e.g., 3.00) usually have zero standard error. Differences that are as large as a full unit may not be significant between groups in different culture bottles despite the small standard errors. However, such large differences between the two sexes of the same cross are significant, and often the sexes differed markedly in a cross but showed no difference by this grading system (e.g., in the genotype $R^{35}(+)/(+)^{21}$ the sexes are of the same grade although they have different phenotypes which do not overlap).

The flies to be graded were collected about every third day and were aged in vials for another four days. The color of the eye changes very little between the fourth and seventh day so that all flies may be assumed to be in a relatively uniform stage of pigmentation. Examination of the eyes was made by a technique discovered late in the course of this experiment and not used during the period of irradiation. It has since been noted that MULLER (1930) found the same technique useful. The flies are placed on a white background in a strong light (as provided by a microscope lamp). By interposing a thin object so that the eye, but not the background directly below it, is in shadow, variegation is strongly accented and other aspects of the coloring are more easily seen. Using a relatively high power of a dissecting microscope allows areas as small as one facet to be distinguished by color, and stocks in which variegation was previously thought to be rare have been shown to be variegated much or all of the time. An impediment to the use of this procedure is that some confusion is caused by rough eye facets, which appear to be variegated. Another cause of error is the process of somatic crossing over. Through the use of an intermediate allele, $(+)^{75}$, which is grade three in color when heterozygous with *bw* and grade four when homozygous, it has been demonstrated that somatic crossing over occurs between brown and the spindle fiber attachment. In $bw/(+)^{75}$ flies, spots attributable to this cause are observed in a little less than one percent of the flies by use of the shadow technique.

The Phenotypic Expression of Brown Locus Position Effects

The position effects which have been analysed in this experiment may always show variegation or be variegated only in some flies; they may deviate markedly in amount of pigment, in some cases overlapping scarlet and in other cases white; they may be of about the same grade in all flies in all cultures or may vary greatly even in the two eyes of the same fly; they may show a strong sex dimorphism in which case the female usually has the darker eye, or no sex dimorphism; they may have a non-random or random distribution of the color within an eye. The above properties of each rearrangement have persisted as long as followed (in some cases over five years). This agrees with the data of other workers that this type of variegation is not of the nature of mutation, but is of the nature of physiological variability. There have been no cases of one of the rearrangements which involves heterochromatin giving an eye color between grade one and grade five which failed to show variegation. Therefore, the partial suppression of pigment is never uniform, although many of the variegated eyes look very much like the non-variegated eyes of the flies carrying combinations of intermediate alleles.

Table 3 sets forth the eye color of a number of these position effects when heter-

TABLE 3

Comparisons of eye color grades

Series / Position effect		A $R^{12}(+)$	A $R^{15}(+)$	(A) $R^{16}(+)$	A $R^{23}(+)$	B $R^{3}(bw)$	B $R^{4}(bw)$	B $R^{17}(bw)$	B $R^{18}(bw)$	C $R^{14}(+)$	C $R^{39}(+)$	C $R^{32}(+)$	C $R^{47}(+)$	C $R^{54}(+)$	C′ $R^{61}(+)$	C′ $R^{57}(+)$	C′ $R^{58}(+)$	C′ $R^{67}(+)$	C′ $R^{73}(+)$
Wild alleles																			
+	♂♂	4.84	4.00	4.72	4.48	4.00	2.76	4.00	5.00	4.00	5.96	3.72	5.00	5.36	4.80	5.24	5.00[c]	3.56	2.00
	♀♀	5.00	4.00	4.92	4.84	4.00	2.12	—	5.00	4.00	5.92	4.56	5.00	5.72	4.92	—	5.00[c]	4.56	2.24
$(+)^{70}$	♂♂		4.28	4.80	5.00	4.48	4.00[c]		5.00	4.00	5.40[c]	4.00	5.00	5.32					
	♀♀		4.04[c]	4.84	5.00	4.00	4.00[c]		4.96	3.80	5.50[c]	4.88	5.00[c]	5.44					
$(+)^{77}$	♂♂			4.48	4.40				5.00[c]			4.07[c]	4.00		4.00				2.05[c]
	♀♀			4.73[c]	4.48				5.00[b]			5.00[b]	4.00		4.88				2.60
Iso-allele																			
$(+)^{21}$	♂♂	4.68[c]	4.00	3.71[c]	4.00	4.00		3.96	4.00	4.00	5.20	2.12	5.00[c]	5.00	3.08	4.96	5.00[b]	2.92	2.00
	♀♀	4.96	4.00	4.00[c]	4.00	3.96		—	4.48	4.00	5.16	3.60	5.05[c]	5.00	4.00	—	4.89[b]	3.00	2.08
Intermediate alleles																			
$R^{24}(+)$	♂♂			3.75[b]	3.91[c]			—				3.00[b]				—			
$(+)^{59}$	♂♂			3.80	4.00	3.84		3.69[c]	3.94[c]	3.00[b]		2.48	4.00[b]		3.72			3.41[c]	2.00
	♀♀			3.68[c]	3.96	3.12		—	3.88[c]	3.00[b]		3.16	4.00[b]		4.08			4.47[c]	2.00[c]
$(+)^{75}$	♂♂			3.68	3.04				3.00[c]				3.00	5.17[b]	3.00[c]			2.67[c]	2.00[b]
	♀♀			4.00[c]	4.00				3.00[c]				3.00	5.67[b]	4.00[c]			3.32	2.00[b]
$(+)^{81}$	♂♂		2.50[b]	4.28	3.88	2.00	1.92	2.00	2.00	1.00	4.56		2.04	4.60	3.96		2.00	3.52	1.36
	♀♀		3.00[c]	4.72	4.96	2.00	0.96	—	2.00	1.00	4.36		2.04	4.88	4.60		2.00	4.10[c]	1.64
Amorphs																			
bw	♂♂	2.84	1.16	2.88	2.60	0.00	0.00	0.00	0.00	0.80	5.48	2.00	1.52	5.00	2.96	4.36		2.00	2.08
	♀♀	3.20	1.80	3.08	3.00	0.00	0.00	—	0.00	0.96	5.36	2.76	1.88	5.46	3.88	—		2.96	2.28
$R^{68}(+)$	♂♂			3.93[c]	4.00[c]			0.00	0.00[c]			3.04						2.12[c]	
	♀♀			4.00[c]	4.92			—	0.00[b]			4.00						3.00[c]	
$(+)^{69}$	♂♂	3.00	1.08	3.64	4.00	0.00[c]		0.00	0.00	0.92	5.00[c]	3.00	1.96		3.56	4.64		2.60	2.00
	♀♀	3.60	1.88	3.64	4.92	0.00		—	0.00	1.13[c]	5.20[c]	3.52	1.80		4.60	—		3.68	2.24
$(+)^{72}$	♂♂	3.60	1.48	3.84[c]	3.52	0.00		0.00	0.00	1.04	5.04	2.00	1.62[c]	4.83[c]	3.28	4.56	1.65[c]	2.04	1.00
	♀♀	3.48	2.00	3.75[c]	4.00	0.00		—	0.00	1.44	4.76	3.00[c]	1.47[c]	4.44	4.00	—	1.64	2.96	1.64
Deficiency																			
$R^{10}(+)$	♂♂			2.00[b]	2.00	[a]		[a]	0.00[c]	[a]			3.00[b]		2.50			[a]	[a]
	♀♀			2.83[b]	2.92	[a]		—	0.00[c]	1.00[b]			2.20[b]		3.00			2.00[b]	[a]
Position alleles																			
$R^{12}(+)$	♂♂	3.50[d]		2.00[b]	1.05[c]	1.00[b]			1.60[c]			1.33[b]	1.00[b]		2.00[b]				
	♀♀	5.00[d]		2.00[b]	1.76	2.00[b]			2.00[c]			2.00[b]	1.60[b]		2.00[b]				

R^{15}(+)	♂♂		[a]		1.00[c]	0.00[b]		[a]	0.50[b]			1.00[b]			1.00[b]				[a]
	♀♀		[a]		1.00[b]	0.33[b]		—	0.67[b]			1.00[b]			1.00[c]				1.00[b]
R^{16}(+)	♂♂			[a]	2.00[c]	2.00[c]		[a]	[a]	[a]		1.00[c]	2.00[b]	2.43	2.00	2.96		1.00	[a]
	♀♀			[a]	3.96	2.00[b]		—	[a]	1.00[b]		2.00[c]	2.00[b]	2.50	2.36	—		1.92	[a]
R^{34}(+)	♂♂				2.00[d]	0.14		1.00[b]	2.00	1.00[b]		1.00[c]	1.00	1.64	1.00			1.00[b]	2.00[b]
	♀♀				3.00[d]	0.12		—	3.00	1.00[c]		1.36	2.00	1.84	2.00			1.88	3.00
R^{3}(bw)	♂♂					[a]	[a]		0.00	[a]		0.20[b]	0.33[b]		0.00			0.00[b]	[a]
	♀♀					[a]	0.00[b]		0.00	0.00[b]		0.25[c]	0.71[c]		0.28			0.17[b]	0.00[b]
R^{4}(bw)	♂♂						0.00[b]			[a]		0.00[b]		[a]					[a]
	♀♀						0.00[b]			[a]		1.00[b]		1.50[b]					[a]
R^{27}(bw)	♂♂							—	[a]	[a]					1.00[b]	—		[a]	[a]
R^{48}(bw)	♂♂								[a]	0.67[b]	3.00[b]	0.77[c]	0.00[b]		1.00		[a]	1.00[b]	[a]
	♀♀								[a]	0.75[b]	2.86	1.05[c]	0.38[c]		1.96		[a]	1.75	[a]
R^{14}(+)	♂♂									[a]		0.90[c]		2.00	1.00[c]	1.00[b]		1.00[b]	[a]
	♀♀									[a]		1.00[c]		2.00[b]	1.00[c]	—		1.00	[a]
R^{20}(+)	♂♂										[a]	[a]						[a]	2.11[b]
	♀♀										[a]	2.57[b]						[a]	2.00[b]
R^{22}(+)	♂♂											2.00[d]	1.00[b]	[a]	1.00	[a]			1.00[c]
	♀♀											2.00[d]	1.83[b]	2.00[c]	1.87	—			1.56
R^{47}(+)	♂♂												2.04	2.67[b]	1.00[b]	2.00			[a]
	♀♀												2.08	2.33[b]	2.00	—			1.67[b]
R^{55}(+)	♂♂													[a]	1.67[c]				2.00[b]
	♀♀													[a]	2.38				2.00[b]
R^{66}(+)	♂♂														1.00	2.00[c]	[a]	1.32[c]	1.00
	♀♀														1.76	—	[a]	2.24	1.84
R^{67}(+)	♂♂															—		[a]	2.00[c]
R^{68}(+)	♂♂																[a]		
	♀♀																[a]		
R^{87}(+)	♂♂																	[a]	1.00[b]
	♀♀																	[a]	2.00
R^{79}(+)	♂♂																		[a]
	♀♀																		[a]

[a] Lethal or strongly semilethal.
[b] Fewer than 10 flies graded. Usually strongly semilethal.
[c] Between 10 and 24 flies graded. Usually semilethal.
[d] Value estimated from descriptive notes. Semilethal.

ozygous with various brown alleles, when crossed with each other, and when homozygous. The upper figure is the average for males, the lower figure for females. The $R^{40}(+)$ deficiency has not been compared with the non-position alleles in table 3 because in aged flies $R^{40}(+)$ is almost identical in action with *bw*. $R^{40}(+)/+$ is lighter than $bw/+$, but to so slight an extent that it should be graded 6.00 under the conditions of this experiment. At eclosion $R^{40}(+)/+$ eyes are of grade 4. This mutant does not cause variegation.

Mutants $(+)^{70}$ and $(+)^{77}$ do not appear to differ from the wild allele in action. On the other hand, $(+)^{21}$, which looks like a wild allele in crosses with point mutations, has a strong tendency toward lowering the color grade of a position effect. It is believed that this mutant represents an iso-allele of the brown locus, although other explanations are possible.

Except for the limitations imposed by the unrelated rearrangement in $R^{24}(+)$, all of the intermediate alleles act like simple point mutations. None of them ever has an effect on eye color when heterozygous with wild type and none ever causes variegation. In the homozygous condition, $(+)^{59}$ appears wild type in color (6.00), $(+)^{75}$ is of grade 4.00 and $(+)^{81}$ is of grade 3.00. $(+)^{24}/(+)^{59}$ and $(+)^{24}/(+)^{75}$ are wild type in appearance, and $(+)^{24}/(+)^{81}$ is of grade 4.00. $(+)^{59}/(+)^{75}$ is of grade 6.00, $(+)^{59}/(+)^{81}$ is of grade 4.00 and $(+)^{75}/(+)^{81}$ is also of grade 4.00. Genotypes $R^{24}(+)/bw$ and $(+)^{59}/bw$ are of grade 4.00, $(+)^{75}/bw$ is 3.00 and $(+)^{81}/bw$ is 2.00.

Of the mutants which have been classified as amorphs, it is possible that $R^{68}(+)$ contributes more to the formation of pigment than the others or than the *bw* allele. This may not be a point mutation, but a position effect, or it may be a point mutation to an allele too weak to make pigment by itself but able to reinforce other alleles. $(+)^{69}$ may be a low grade of intermediate allele too, although this is less likely. $(+)^{72}$ appears to be identical with *bw*.

There does not seem to be a strong correlation between the position of the break in region 59 and the phenotype which it produces. However, mutants $R^{20}(+)$, $R^{55}(+)$ and $R^{57}(+)$ are very close in phenotype and are breaks at or near the same point. These three are the only position effects which clearly have an eye color greater than grade 4.00 in the genotype $R(+)/bw$, and this genotype is marked, only in these three stocks and $R^{12}(+)$, by a very low correlation between the two eyes of a single fly. It is not unusual to find flies in these stocks with one eye almost completely pigmentless while the other eye can be distinguished from wild type with difficulty, if at all. The position of the break toward the left of 59D cannot be the distinguishing factor since $R^{67}(+)$ is to the left of these three and is light in color, and the mutant $R^{50}(+)$, which was also broken to their left, was one of the lightest pigmented forms. No other cases of a relationship between a break and eye color have been seen.

Table 3 brings out the lighter pigmentation of each of the position effects when heterozygous with *bw*, indicating relationship with the brown locus, except for $R^{79}(+)$. Actually, there is only a small amount of overlap between the light pigmentation of $R^{79}(+)/bw$ and the slightly darker color of $R^{79}(+)/+$, but the variation is almost entirely within grade 2 and so does not show on this grading scale. A finer grading scale, which has been used, did show a distinct difference. Although

the white color of the $R(bw)/bw$ combinations could be explained as something other than a position effect at the brown locus, it is most likely that all of them are affected primarily at the *bw* locus.

Because purpleoid is close to the brown locus, almost all of the position alleles were crossed to it for possible indications of interaction. $R^{79}(+)$, which is the mutant with the break closest to the presumed position of purpleoid, seemed to show a more highly pigmented heterozygote with purpleoid than with wild, but this has not recurred upon retesting. At any rate, such an interaction would not have been expected. No other mutant which was tested with purpleoid gave an unusual result.

Lethality

Lethality or strong semilethality is shown, particularly in the right-hand part of table 3, by the letters *a*, *b* or *c*. Because most of these crosses had to be made up in such a way that a number of marked flies of other genotypes also occurred, it was possible to get an estimate of the expected number of R/R heterozygotes. In most instances the deficiency of R/R individuals was so great as to leave no doubt of the inviability of the genotype. It will be noticed in table 3 that in a number of crosses the males proved to be less viable than the females.

If the lethality of R/R heterozygotes is compared with the position of the break in region 59, it will be seen that there is evidence that the closer two breaks are to each other, the more likely they are to produce a lethal heterozygote. Thus, $R^{79}(+)$ is inviable with all four of the nearest mutants, $R^{18}(+)$, $R^{4}(bw)$, $R^{45}(bw)$ and $R^{14}(+)$, but it is partially viable with the four most distant mutants, $R^{20}(+)$, $R^{55}(+)$, $R^{57}(+)$ and $R^{67}(+)$. The other position alleles which are at the ends of the space in which breaks occur also show some effects of greater lethality with neighboring breaks than with distant breaks. The mutants which are most viable as homozygotes, $R^{12}(+)$, $R^{35}(+)$, $R^{32}(+)$, $R^{47}(+)$ and $R^{56}(+)$, are found in the center of the area in which breaks in region 59 occur. This distribution does not appear to be random. It is possible that the lethality which is shown is due largely to a highly sensitive lethal locus toward each end of the 59D to 59F region, although lethal loci less sensitive to position effects may be distributed throughout the region, or lethality may be due in part to the other breaks which are involved.

In those R/R genotypes which are semilethal, even when adult flies do occur they are often deformed. The deformities include small bristles, small eyes, incompletely expanded wings, and melanotic protuberances and inclusions. This melanotic condition is probably the same as that described as "melanotic clots of dried blood" in the heterozygotes between bw^{VDe3} and the position allele bw^{5} (BRIDGES and BREHME 1944). Only one of the homozygous position effects, $R^{47}(+)/R^{47}(+)$, has shown this phenotype strongly. The melanotic material may be internal or external or both, and if external it may bear a small amount of non-melanotic tissue at the tip on rare occasion. The melanotic condition is noted most frequently just posterior to the proboscis. Infrequently, a fly with one chromosome of the normal arrangement will show the melanotic condition. The malformed flies are less viable than normal and often fail to emerge from the pupal case. A few of the genotypes which are listed as lethal are able to advance to this late pupal stage.

DISCUSSION

The Reciprocal Position Effects R(+)/bw and R(bw)/+

EPHRUSSI and SUTTON (1944) divide the interpretations of the position effect into two categories, kinetic, or chemical diffusion theories, and structural theories. An extensive structural theory is developed in their paper. This theory traces to the suggestion by MULLER (1938, 1941) that synaptic forces, and especially somatic pairing, which is found in the Diptera, are related to the activity of the gene so that a change in pairing might be accompanied by a change in gene action. On this assumption, the structure *R*(+)/*bw* is equivalent to *R*(*bw*)/+. This equivalence should be observable (1) in the rate at which position effects can be induced, and (2) in the phenotypes produced by position effects.

The earliest exception to the rule of equivalence was in the work of DUBININ and SIDOROV (1935) on the hairy locus. They found the deviation from wild type was greater in *R*(+)/*h* than in *R*(*h*)/+. The rearrangement was the same in both cases, the difference having been obtained by finding the rare crossovers which occurred between hairy and the break. STERN, SCHAEFFER and HEIDENTHAL (1946) found position effects at the cubitus interruptus locus to be formed more frequently as *R*(+)/*ci* than as *R*(*ci*)/+. The difference in rate of formation is highly significant. The *R*(+) alleles were not separated into those associated with rearrangements and those due to mutation, but evidence is presented to show that at this locus mutation is a rare process when compared with rearrangement. A distinction in phenotypes is also shown as *R*(+)/*ci* deviates from wild type to a significantly greater extent than *R*(*ci*)/+.

As at the cubitus interruptus locus, the brown locus forms (+)/*bw* alleles more frequently than (*bw*)/+ alleles. However, this difference is not statistically significant if the comparison is restricted to position effects. Still, it should be noted that the deviation from equality is in the same direction as that found for cubitus interruptus, namely, that the wild allele has formed more position effects than the recessive. The phenotypes also differ between *R*(*bw*)/+ and *R*(+)/*bw* stocks. Of the six *R*(*bw*) mutants which have been examined in this experiment, none have included any *R*(*bw*)/+ individuals with an eye color as light as grade zero or one. Only R^{45}(*bw*)/+ appears to have representatives which have patches of pigmentless facets. Of the *R*(+) mutants, only those occurring in the *C* or *C′* series can be considered, since the *A* series mutants may have been selected for a high degree of expression of the position effect. Eight of the twelve variegated position effects studied in the *C* and *C′* series have *R*(+)/*bw* individuals as light as grade zero or one. All four of the other position effects occasionally show groups of pigmentless facets. Three of the sterile mutants in these series were noted as having eyes of grade one but no sterile mutant individual in the *B* or *B′* series was noted as having eyes with any pigmentless areas. The difference between none of six *R*(*bw*)/+ position effects producing individuals as low as grade one and eight of twelve stocks ranging this low in the comparable *R*(+)/*bw* series can be determined statistically by a slight modification of the exact method described by FISHER (1950). The chance of getting this deviation is 0.011, or a probability of 0.022 if this great a

deviation in both directions is considered. If the production of pigmentless patches in the eye is considered, the distribution five to one in the *B* series and zero to twelve in the *C* and *C'* series has a probability of random occurrence of 0.0014.

Heterozygotes Between the Variegations and the Deficiency

$R^{40}(+)$ may be the only case of a dominant eye color associated with a deficiency. It was found in this experiment because of the pseudodominance of the *bw* allele and the favorable conditions of observation presented by the use of scarlet. The dominant eye color is best seen immediately after eclosion.

DUBININ and SIDOROV (1935) crossed their position effect for hairy with a deficiency for the locus and found that the heterozygotes with the deficiency were more normal than the heterozygotes with the recessive. STERN, MACKNIGHT and KODANI (1946) have obtained heterozygotes between four $R(+)$ alleles and a deficiency which includes the cubitus interruptus locus. The phenotype of these heterozygotes was almost normal although the phenotype of comparable $R(+)/ci$ combinations was highly abnormal.

The comparison of $R/R^{40}(+)$ with R/bw heterozygotes shows an effect of $R^{40}(+)$ which varies from a strong reduction in pigment to one case of an increase in pigment. The number of individuals observed is small in the latter case and it is possible that the moderately colored eyes of $R^{47}(+)/R^{40}(+)$ represent a chance deviation from the lighter eye color of $R^{47}(+)/bw$. There is certainly no strong tendency for this deficiency to shift position effects toward normality.

Homozygous Position Alleles

The phenomenon of reconstituting wild type in the homozygote, which was reported by DUBININ and SIDOROFF (1934) for the cubitus interruptus locus, and which is further discussed by STERN (1948), is not present in this brown locus material. Except in the case of $R^{12}(+)/R^{12}(+)$, which is approximately identical with $R^{12}(+)/+$, all of the homozygotes for $R(+)$ alleles are much lighter than the comparable $R(+)/+$, but they may be lighter or darker than $R(+)/bw$. No general rule has become manifest. The two $R(bw)$ homozygotes which have been observed, $R^{4}(bw)$ and $R^{25}(bw)$, were pigmentless, as was expected. The $R(+)$ homozygotes are much darker than those which were studied by GLASS (1933), three of whose $R(+)$ homozygotes can be classified as grade one (or possibly including some grade two individuals) and another of which is described as without pigment, or grade zero. Only the $R^{56}(+)$ homozygote may overlap with these. The difference may be that the favorable conditions for the selection of mutants in this experiment detected position alleles causing smaller deviations from wild type.

Heterozygotes for Two Variegated Position Alleles

Crosses between variegated position alleles have rarely been made. VAN ATTA (1932) found no viable R/R heterozygotes between six different brown position alleles which were crossed in all possible combinations. GLASS (1934) crossed a number of brown position alleles with each other and noted that the R/R heterozygotes were rare and poorly viable. The eye color was described as being similar to

that found in homozygotes for the same position effects, or probably grades zero through two. Remarks in BRIDGES and BREHME (1944) (see bw^{V1} and bw^{VD}) indicate that other crosses of brown position alleles have been carried out, but the phenotypes of the R/R heterozygotes have not been given. DUBININ and SIDOROFF (1934) reported that $R(+)/R(+)$ heterozygotes are wild type at the cubitus interruptus locus. At the same locus, STERN and HEIDENTHAL (1944) found that $R(+)/R(ci)$ gives more normal wing venation than $R(+)/ci$. In fact, the $R(+)/R(ci)$ heterozygotes were more normal than some of the comparable $+/R(ci)$ phenotypes though less normal than others.

It has been found in this experiment that $R(bw)/R(bw)$ heterozygotes produce no pigment. The average grade of $R(+)/R(bw)$ heterozygotes is frequently less than 1.00 and always less than both the comparable $R(+)/bw$ and $R(bw)/+$ heterozygotes. The $R(+)/R(+)$ heterozygotes are less pigmented than either $R(+)/+$ (assuming that the value for the $R^{85}(+)/R^{79}(+)$ males is accidentally high), and usually have even less pigment than either $R(+)/bw$ heterozygote. The general rule may be stated that the pigment formed in $R(+)/R$ heterozygotes is correlated with the pigment formed by the $R(+)$ alleles in the heterozygote $R(+)/bw$, and, where applicable, with the pigment occurring in $R(bw)/+$ heterozygotes. The numbers of individuals in table 3 are often small and inversions of order are present, but the rule holds good as an approximation.

There are several mechanisms by which a chromosomal rearrangement may affect the action of a gene. It may directly affect the gene's ability to perform its function or it may affect either its substrate or product, or it may operate through a combination of these. These data do not permit a distinction to be drawn between the various modes of action. The change in phenotype which appears to be due to a rearrangement on the chromosome carrying a particular gene will be referred to as an "intrachromosomal effect," while the change in phenotype which appears to be due to a rearrangement on one chromosome affecting the gene on the homologue will be referred to as an "interchromosomal effect." These terms have been chosen for simplicity and they may not accurately describe the processes which are taking place.

Since the *bw* allele can be assumed to be an amorph, the pigment produced by the genotype $R(+)/bw$ can be assumed to be due solely to an intrachromosomal effect of the $R(+)$ condition, and as such is an exact measure of this intrachromosomal effect. On the other hand, there is no reason to believe that pigment is being formed by an $R(bw)$ chromosome, and therefore the pigment of an $R(bw)/+$ individual may be interpreted as a measure of the interchromosomal effect of the $R(bw)$ rearrangement upon the normally located allele. The combination $R(+)/R(bw)$ owes its pigmentation solely to the pigment which is produced by the chromosome carrying the wild allele, but the amount of pigment which is produced by this complex is determined by the combination of the intrachromosomal effect of the $R(+)$ and the interchromosomal effect of the $R(bw)$ rearrangements.

Two separate theories may be postulated for the interaction of position alleles. The first of these theories might suggest that the interaction of two position alleles is dependent upon factors such as the point of breakage and the region to which the

locus has been transposed. Under this theory the phenotype of an R/R heterozygote could not be predicted without an extensive knowledge of the action of each of the rearrangements in many such combinations. Opposed to this is the theory that the interchromosomal and intrachromosomal effects of any rearrangement are constant. On this theory if one could determine the effect in one combination of a particular position allele, this could be used for an accurate prediction of the effect of this position allele in other combinations.

The single interchromosomal plus intrachromosomal effect found in the $R(+)/R(bw)$ heterozygotes affords a simple test of these theories. According to the second theory if $R^{m}(bw)/+$ is lighter than $R^{n}(bw)/+$ then $R^{m}(bw)$ should form lighter heterozygotes with any particular allele than $R^{n}(bw)$ forms. Conversely, if $R^{a}(+)/bw$ is lighter than $R^{b}(+)/bw$, then $R^{a}(+)$ should form lighter heterozygotes with any particular $R(bw)$ allele than $R^{b}(+)$ forms. The first theory of interaction would predict that these rules do not hold and that the relative pigmentation of an $R(+)/R(bw)$ heterozygote could not be predicted from a knowledge of the action of these two position alleles in combination with non-position alleles.

Among the $R(bw)$ alleles we may assume the order $R^{4}(bw)$, $R^{3}(bw)$, $R^{27}(bw)$, $R^{45}(bw)$ as the sequence from light to dark, and accidents of sampling could be responsible for the slightly light values of the combination of $R^{27}(bw)$ with $(+)^{21}$ and $(+)^{59}$, and the slightly dark combination of the females of $R^{4}(bw)/R^{32}(+)$ (which figure is based on only five individuals). The great number of observations made with $R^{3}(bw)$ and $R^{45}(bw)$ allows a more critical investigation to be made of the relationship between these two position alleles. $R^{45}(bw)$ is consistently as dark or darker than $R^{3}(bw)$ with the exception of the cross of these rearrangements with $R^{47}(+)$. A reinvestigation of the amount of pigment formed by $R^{45}(bw)/R^{47}(+)$ shows that the original observations were accidentally too low and the values for each sex are probably in the neighborhood of 0.80, which would put these figures in line with those for other crosses. With respect to the estimation of the intrachromosomal effect of $R(+)$ alleles from their combination with *bw*, it may be stated that the $R(+)/R(bw)$ heterozygotes give remarkably good agreement with the predictions considering the small numbers which are frequently involved. The minor inversions of order which do occur may be due in part to the fact that viability and amount of pigment seem to be positively correlated. It has been noticed in certain genotypes that flies with greater than average pigmentation were often normal in most other respects although their sibs were quite defective. If a genotype is almost completely lethal, as is $R^{3}(bw)/R^{47}(+)$, the few flies which do emerge as adults are not likely to be a random sample of the genotype with respect to eye color.

These facts strongly suggest that the interaction of position alleles in combinations between two of them is neither related to the relative positions of the breaks involved or to an interaction dependent upon the materials which have been transposed to the neighborhood of the brown locus, but rather that there is a simple intrachromosomal effect and a simple interchromosomal effect which should be calculable for each position effect and that these effects combine in some direct manner to produce the pigment which is formed. It has been noted that an $R(bw)$ position allele has no intrachromosomal effect and an interchromosomal effect can be calculated by

reference to the pigment of $R(bw)/+$ flies. The intrachromosomal effect of $R(+)$ alleles can be calculated from the pigment of $R(+)/bw$ flies and a measure of the interchromosomal effect can be obtained from a study of $R(+)/+$ flies, although this is greatly complicated by the presence of the intrachromosomal effect.

The greatest difficulty besetting an adequate analysis of these data is probably the fact that the scale on which pigment was graded is not linear and the depart ure from linearity are completely unknown. Thus, these data are inadequate for the estimation of whether the difference between grades one and two is of the same order of magnitude as the difference between grades two and three or three and four.

Theories Explaining the Position Effect

These findings suggest that $R(+)/bw$ position effects are formed at a greater rate than $R(bw)/+$ position effects, and it is highly probable that the former may occasionally have less pigment formed in their eyes. Therefore, it seems reasonable to assume that the two genotypes are not identical in nature, and intrachromosomal effects are stronger than interchromosomal effects. To this may be added much of the unequivocal evidence compiled by STERN and his coworkers, and the evidence relating to the hairy locus. All of these facts argue against the simple structural theory of EPHRUSSI and SUTTON. The author would like to note that at the time that this work was begun the structural theory seemed to be the most plausible one. It covered and explained almost all of a multitude of different observations which were known at that time. Furthermore, it could be used to predict results, which indeed was what prompted the work reported here.

The structural theory has two basic premises, (1) that heterozygosity in chromosomal structure is equally effective in disturbing the action of the genes on the rearranged and on the normal chromosomes, and (2) heterochromatin, and on rare occasion an unusual distribution of euchromatin, may cause a disturbance in the action of the gene even though there is homozygosity of structure. Most of the observations of this paper could be accounted for by the additional assumption that in a structurally heterozygous form which is influenced by heterochromatin the effect is greater on the chromosome which contains the heterochromatin than on the chromosome which is normal.

Even this additional hypothesis does not completely remove all difficulties. For example, $R^{56}(+)/R^{56}(+)$ is much lighter in eye color than $R^{56}(+)/bw$, although it is obvious that the position allele is contributing to pigment formation while the recessive allele is not. It would appear in this case that there is much less effect of heterozygosity of structure than of heterochromatin. Another argument against the theory that structural heterozygosity produces the position effect is the fact that almost no position effects due to a rearrangement which does not involve chromocentral heterochromatin have been found at any locus. Even if the brown locus is more sensitive to heterochromatin than it is to euchromatin, at least a few euchromatic position effects other than the deficiency, $R^{10}(+)$, should have been detected in this experiment. In fact, it appears that the deficiency is the only known euchromatic position effect which involves the brown locus (HINTON and GOODSMITH 1950, refer to brown-dominant as a "band of heterochromatin", and new

cytological evidence plus genetic evidence such as the production of variegation will be presented elsewhere as proof of the heterochromatic nature of this mutant). Furthermore, the structural theory would require that the pigmentation of heterozygotes between two position alleles would not be predictable except with reference to the site of breakage and the material which has been transposed to the brown locus, and it seems quite likely that this is not so. From the above facts, it appears that the structural theory is neither predicting the action of the brown locus nor explaining the facts which have been observed.

The pertinent facts which have been observed about the brown locus are the following:

(1) there is a simple dominant-recessive relationship between the alleles *bw*$^+$ and *bw*,
(2) a particular deficiency for the locus has been found to have a slight dominant effect,
(3) a rearrangement which brings heterochromatin near the locus decreases the amount of pigment formed,
(4) substitution of $R(+)$ for $+$ or substitution of $R(bw)$ for *bw* usually decreases and never increases the amount of pigment formed,
(5) $R(+)/bw$ tends to deviate further from normal in phenotype than $R(bw)/+$.
(6) it is probable that each position allele has a distinct intrachromosomal effect and a distinct interchromosomal effect and that these are not dependent upon the structure of the homologous chromosome.

We may assume that *bw*$^+$ is an active allele and that *bw* is inactive. This is warranted by the general consideration of gene action and by the evidence of the mutants which were formed in the course of these experiments. The position effect at the brown locus can be explained by the further assumption that heterochromatin is able to interfere with the production of pigment by the wild allele. The effect of heterochromatin must be greater on the chromosome which contains it than on the homologue, but it must act on both chromosomes. It is possible that changing the euchromatic loci near the brown locus can affect *bw*$^+$ to a lesser extent, which would explain the action of the dominant deficiency, $R^{40}(+)$. An alternative explanation for $R^{40}(+)$, which would require that the wild allele at the brown locus is not a complete dominant, is not too likely since a number of recessive mutants were obtained in these experiments but the deficiency was the only one which was detected by a slight dominant effect. It is probable that the majority of the recessive alleles which were induced in the course of these experiments were amorphs with no greater output of brown locus product than the deficiency.

The fact that we do not know the position of most loci on the salivary gland chromosomes to within a few bands and that the position of the centromeres is hazy leaves the word "heterochromatin" playing a major role which it may not deserve. There is nothing in these experiments which would indicate that it is not proximity to the centromere rather than to the heterochromatin associated with it which is the cause of the position effect. However, the mutant brown-dominant, which is due to heterochromatin which is divorced from the centromere, indicates that heterochromatin is truly the agent.

SUMMARY

Irradiation of *bw*$^+$ males probably produced 7 position effects among 34,682 flies when mated with *bw*$^+$ and 23 position effects among 61,385 flies when mated with *bw*. Irradiation of *bw* males probably produced 12 position effects among 50,453 flies when mated with *bw*$^+$ and 0 among 22,835 when mated with *bw*. The rate of formation of $R(+)/bw$ is not significantly higher than the formation of $R(+)/+$ or $R(bw)/+$. Among the position effects examined, the amorph phenotype in some facets of the eye was found in all twelve $R(+)/bw$ stocks. Only one of the six $R(bw)/+$ stocks showed this phenotype. This deviation from random would occur about once in a thousand times. In addition to the position effects, the $(+)/bw$ flies included one iso-allele, four intermediate alleles, a deficiency and many amorphs. The other crosses did not produce any mutants of these types.

The deficiency has a slightly dominant position effect associated with it. It is the only euchromatic position effect associated with *bw*. All of the variegated position effects are due to chromosomal rearrangements involving 59D to 59F and the chromocentral heterochromatin of the Y, 2, 3 or 4. The X chromosome may have a lower ability to form position effects. There may be one group of three position effects which shows a relationship between eye color phenotype and the position of the break. There is a clear connection between lethality and the position of the break. Heterozygotes between two breaks which are close together are more likely to be lethal than heterozygotes between breaks which are far apart. A lethal locus sensitive to breaks in its neighborhood may be present toward each end of the *bw* sensitive region in 59.

A few of the mutants are viable as homozygotes, with phenotypes of $R(+)/R(+)$ as dark as $R(+)/+$ or lighter. One $R(+)$ homozygote is much lighter than $R(+)/bw$. Heterozygotes between two position alleles, at least one of which is $R(+)$, are related in color to the color of the $R(+)/bw$ or $R(bw)/+$ heterozygotes involved. Substitution of $R(+)$ for $+$ or of $R(bw)$ for *bw* either does not affect or else reduces pigmentation, but never increases it. The position alleles appear to have distinct intrachromosomal and interchromosomal effects and it appears that the amount of pigment which is formed by heterozygotes for two position alleles can be predicted from a knowledge of the amount of pigment formed by each allele when in combination with non-position alleles.

The structural theory of position effect does not explain these facts satisfactorily. A simple chemical theory which assumes that the *bw* locus is slightly affected by euchromatic rearrangements but strongly affected by heterochromatin is advanced. The heterochromatin can affect both chromosomes, but it appears to act more strongly on the chromosome which contains it than on the normal homologue.

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SPERMATOGONIAL EXCHANGE BETWEEN THE X AND Y CHROMOSOMES OF DROSOPHILA MELANOGASTER[1]

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ALTHOUGH meiotic crossing over has not been demonstrated to occur in the male of *Drosophila melanogaster*, recombinant chromosomes are recovered from males with a very low frequency. Since such recombinants often occur in clusters of individuals among the progeny of a single male, they have generally been attributed to exchange occurring during gonial mitosis. The origin of recombinants from males has been discussed by COOPER (1944).

Recombinants of the above type involving exchange between the X and the Y chromosomes are of particular interest. Such exchange implies homology, and that the X and Y are in part homologous is well established; little can be said with respect to the extent or the arrangement of the homologous regions, however. It is known that these exchanges are confined to the heterochromatic regions of the X and Y; the heterochromatin of the X is located in the region of the centromere, and the Y is completely heterochromatic. The complete analysis of products of exchange between X and Y is generally hampered by the paucity of genetic markers in the heterochromatic regions, even leading to an ambiguity in some cases as to which arm of the Y chromosome was involved. The experiments to be discussed here were designed to obtain more specific information on the homologies between the heterochromatic regions of the X chromosome and the Y chromosome.

EXCHANGE BETWEEN THE Y CHROMOSOME AND THE sc^8 INVERSION

In 1940 and 1941 SIDOROV reported results which he interpreted as the consequence of exchange between the short arm of the Y chromosome and the heterochromatin shifted to a distal position by In(1)sc^8 (see figure 1). Among the progeny of In(1)sc^8 males crossed to *y* sc^{13} w^a females he found, in addition to the regular classes, occasional *y* females and sc^{13} w^a males. The *y* females could be shown to carry In(1)sc^8 deficient for the distal uninverted euchromatic section. Of seven such chromosomes tested, six were found to carry all of the male fertility factors normally found on Y^S, whereas none carried all those of Y^L. These chromosomes then were interpreted as recombinants in which the distal euchromatic region of In(1)sc^8 had been replaced by Y^S through heterochromatic crossing over. The sc^{13} w^a males were sterile and were presumed to carry the reciprocal product, a Y chromosome in which part of Y^S had been replaced by y^+ and ac^+ from sc^8. To test this, In(1)sc^8 males were crossed to

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FIGURE 1. Schematic representation of the event pictured by SIDOROV (1940 and 1941) as giving rise to exceptional progeny from crosses involving In(1)sc^8 males. The heavy lines represent heterochromatin.

attached X females homozygous for *y v f*; the non-yellow daughters recovered from this cross were shown to carry the predicted recombinant type. CREW and LAMY (1940) have recovered a similar recombinant Y from In(1)sc^{81}.

Two aspects of the above phenomenon seemed particularly to merit further investigation. First, any exchange between the Y chromosome and an inverted segment of the X chromosome which does not result in the formation of a dicentric would seem, on preliminary consideration, to require an unusual pairing arrangement in which the linear order of one homologue is inverted with respect to that of the other. Secondly, the apparent preferential involvement of Y^S in the exchange might further elucidate the distribution of the regions of X-Y homology. Consequently, experiments were set up in an attempt to repeat SIDOROV'S observations.

Recovery of reciprocal recombinant types, for further genetic study, from a single cross requires that one of the products be recoverable in a fertile male; it will be noticed that in SIDOROV'S experiments recovery of testable products was possible in daughters alone. Viability and fertility of males carrying the deficient sc^8 chromosome (recombinant X chromosome) or the deficient Y chromosome (recombinant Y chromosome) can be assured by supplying the *y-ac* region or the proper Y fertility factors respectively. Crosses employing each of these methods were used in the experiments described below.

Experiments involving In(1)sc^{8L},EN^R males

The composite chromosome, In(1)sc^{8L},EN^R, differs essentially from In(1)sc^8 used by SIDOROV in that it carries at the right end (centromere end) all of the euchromatic material from the left end of a chromosome in normal sequence that is necessary for survival; consequently a deficiency for the distal sc^8 euchromatin is compensated for by a duplication of this material proximally. Furthermore, since the proximal yellow locus carries a mutant allele, recombinant X-bearing males are yellow. The nature of the proximal heterochromatin of In(1)EN is largely unknown; there is a heterochromatic short arm about one-fourth the length of the long arm. For a fuller description of In(1)EN, see NOVITSKI (1949).

Two series of crosses were run in which In(1)sc^{8L},EN^R, y^+ *f y* males were mated individually to attached X females carrying yellow plus other markers. In the first series (203 pairs) *y w*/Y females were used, and the parents were kept for two five-day egg laying periods; in the second series (47 pairs) *y^2 su-w^a w^a bb*/Y females were used, and the parents were transferred through five five-day egg laying periods. The results of the above crosses are summarized in table 1.

TABLE 1

Progeny of In(1)sc^{s}L,EN^{R}, y^{+} f y/Y ♂♂ × y w/Y ♀♀ (Experiment 1) and In(1)sc^{s}L,EN^{R}, y^{+} f y/Y ♂♂ × y^{2} su-w^{a} w^{a} bb/Y ♀♀ (Experiment 2)

Experiment	y^{+} males	y males	y females	y^{+} females
1	33,527	19	31,723	5
2	5,028	14	4,436	0

Of the 19 yellow males recovered in experiment 1, five were offspring of one parental male (F-7), four were offspring of another (D-6) and one was recovered from each of ten other males (B-1, C-4, I-7, O-7, P-7, P-8, Q-1, and U-5). In experiment 2 eleven yellow males were offspring of one parental male (C-1) and one was recovered from each of three others (B-7, C-6, and C-13); such clustering was not observed by SIDOROV. The non-yellow females arose singly (L-7, U-7, U-8, V-0, and V-4). In view of the rarity of recombinants, it is justifiable to consider clusters as arising through cell division from a single mitotic event. Henceforth the recombinant X chromosomes will be abbreviated sc^{s} EN c.o. X, and the recombinant Y chromosomes sc^{s}EN c.o. Y.

The constitution of most of the above exceptions plus others (a total of 37) recovered from similar crosses was tested with respect to Y chromosome fertility factors. This was accomplished by putting each recombinant into males in combination with the fertility factors of one arm or the other of the Y chromosome and testing the fertility of the resulting individuals. Such males, when fertile, were concluded to carry, as part of the recombinant chromosome, the fertility factors of the Y arm not supplied. Fertility of the sc^{s}EN c.o. X's was tested in combination with Y″(= Y^{S}·Y^{S}—STERN 1929) and Y^{cl} (= Y^{L} closed—MULLER 1948), while that of the sc^{s}EN c.o. Y's was tested in the presence of essentially reciprocal attached X breakdown products, X·Y^{S} and X·Y^{L} (the elevated dot indicates centromere position).

Of thirty-seven sc^{s}EN c.o. X's tested, four were fertile in males in the presence of Y^{cl} (L-7, P-0, P-7, 35a), while none of them were male fertile in the presence of Y″. Since these thirty-seven cases include all members of clusters F-7, D-6, and C-1, it can be further stated that involvement of the Y chromosome was demonstrated in four of twenty events from which exceptional X-bearing progeny were recovered. Of the five sc^{s}EN c.o. Y's, three were tested; all of these were found to confer fertility upon X·Y^{S} males but not X·Y^{L} males. Adding these cases to the recombinant X class it is found that seven of twenty-three events studied can be demonstrated to have involved the Y; furthermore all seven involved Y^{S}.

Experiments involving In(1)sc^{s} males

In these experiments In(1)sc^{s} males were crossed to Y^{S}X·Y^{L} females homozygous for *y*; the recombinant X chromosomes were therefore recovered as *y* females, and the recombinant Y chromosomes were recovered as *y*$^{+}$ males which had received a Y^{S}X·Y^{L} chromosome from their mother and were thus fertile irrespective of the paternal Y contribution

Experiments 1 and 2 have demonstrated the occurrence of clusters of recombinants

to be expected from mitotic exchange; it was deemed desirable to recover such clusters in as many cases as possible, with the hope of recovering reciprocal types from the same father. With this in mind, the two experiments described below have been designed to encourage maximum yield of offspring from individual males. In experiment 3, 25 In(1)sc^{8}, *f v cv sc*^{8} males were mated individually to five females of the constitution Y^{S}X·Y^{L}, In(1)EN, *B y*. At the end of five days the males and females were separated; each male was placed in a new bottle with five fresh females of the same constitution, and the old females were put into new bottles for continued egg laying. After five more days the bottles containing females alone were transferred and the bottles containing one male and five females were subjected to the same procedure as described for the first series of bottles. At the end of the next five days the procedure was repeated except that the males were discarded; at this time there were theoretically nine bottles from each of 25 parent males. Experiment 4 was arranged in a similar manner except that only four females per male were used and subculturing was done every four days. Also it was discovered in experiment 3 that females, once removed from the males had sperm enough for only two additional subcultures; in experiment 4, therefore, females were discarded after the second subculture. In this case transfers were continued to the point where for each of 100 parental males there were maximally 14 bottles.

Careful examination of the cross used in experiment 3 reveals that the recombinant X chromosomes (sc^{8} c.o. X) are recovered as the heterozygote, Y^{S}X·Y^{L}, In(1)EN, *B y*/sc^{8} c.o. X. These two chromosomes have essentially the same sequence; consequently crossing over is expected to occur freely between them. Crossing over distal to *B* results in exchange of tips which is undetectable in terms of the markers employed. Recovery of such a recombinant between Y^{S}X·Y^{L} and sc^{8} c.o. X in the process of establishing stock of a sc^{8} c.o. X chromosome would result in the loss of the critical distal region, which may or may not carry Y^{S}, and in its replacement by the Y^{S} tip from the Y^{S}X·Y^{L} chromosome. Actual measurements of the rate of recombination between *B* and the tip in such a heterozygote yield a value of 21.9%. To assure the recovery of the proper chromosome end in experiment 3, separate stocks were made and maintained for all further testing from three sons of each heterozygous female recovered. To eliminate the necessity of establishing more than one stock of each recombinant in experiment 4, the In(1)dl-49, *car f v* section of an Ins(1)sc^{8},dl-49, *car f v* chromosome, kindly supplied by Dr. H. J. Muller, was transferred into the Y^{S}X·Y^{L} chromosome by a double crossover. It was anticipated that the presence of In(1)dl-49 would practically eliminate crossing over distal to the inversion, but when test counts were made it was discovered that the rate of recombination between *car* and the tip was 9.3%, that between *f* and the tip being 20.9%; this is an extraordinarily high rate of crossing over for a dl-49 heterozygote and it may be surmised that the heterochromatin placed distally in the two sequences may be responsible. That the dl-49 inversion was actually present in the cross was confirmed by salivary gland chromosome observations. Since the presence of In(1)dl-49 had little or no effect on crossing over, it was also necessary in experiment 4 to establish several stocks of each recombinant X chromosome (four or five).

The results of experiments 3 and 4 are summarized in table 2. In experiment 3 two

TABLE 2

Progeny of In(1)sc^{s}, f v cv sc^{s}/*Y* ♂♂ × *Y^{S}X·Y^{L}, In(1)EN, B y* ♀♀ *(Experiment 3) and In(1)sc^{s},* f v cv sc^{s}/*Y* ♂♂ × *Y^{S}X·Y^{L}, Ins(1)EN, dl-4g,* car f v y ♀♀ *(Experiment 4)*

Experiment	y^+ females	*y* females	*y* male	y^+ males
3	11,381	3	8,085	3
4	93,675	6	85,818	0

of the *y* females and one of the y^+ males were among the offspring of one parent male (E_1), while the rest of the exceptional individuals occurred separately (sc^{s} c.o. X P_0, sc^{s} c.o. Y Q_2, sc^{s} c.o. Y T_0). In experiment 4 two *y* females came from one father (67h), and the remaining four came from separate fathers (89a, 25b, 91b, 99c).

Tests carried out on the sc^{s} c.o. X chromosomes to demonstrate the presence of Y chromosome fertility factors were the same as those employed in the case of sc^{s}EN c.o. X's except that the males tested for fertility carried Dp(1;3)sc^{f4} in addition to the recombinant X and Y$''$ or Y^{cl}. This duplication, which carries the tip of the X chromosome, through the locus of *sc*, on the extreme left end of chromosome 3, is necessary to cover the deficient euchromatic region of the sc^{s} c.o. X. The three sc^{s} c.o. X chromosomes from experiment 3 (E_1a, E_1b, P_0) were found to carry Y^{S} fertility factors while two of the recombinant Y's (Q_2 and T_0) were found to lack Y^{S} fertility factors. From experiment 4 three chromosomes were found to carry Y^{S} fertility factors (89a, 91b, 67h(2) but not 25b, 99c or 67h(1)). In no case was the presence of Y^{L} demonstrable.

POSSIBLE MODES OF Y INVOLVEMENT

The recombinant X chromosomes recovered from males carrying the distal portion of In(1)sc^{s} cannot always be shown to involve the Y chromosome. The remainder of this paper, however, will concern itself with only those recombinants which can be demonstrated to have definitely involved the Y. The question arises as to whether the methods employed in the detection of Y arm involvement were by any means exhaustive; let us consider therefore the possible pairing configurations between the distal heterochromatin of In(1)sc^{s} and the Y chromosome and the outcome of exchange in each case. These configurations are shown in figures 2 and 3 for the case of In(1)sc^{sL},EN^{R}, y^+ *f y* males; these figures will suffice also for the case of In(1)sc^{s} males if the differences between the proximal ends of In(1)sc^{s} and In(1)EN are kept in mind. In the following treatment these differences will be pointed out wherever they influence the argument. In figures 2 and 3 Y^{S} and Y^{L} have not been labelled as such; instead the more general terms synaptic arm (Y^{syn}) and asynaptic arm (Y^{as}) have been employed (either Y^{L} or Y^{S} could be either synaptic or asynaptic). The synaptic arm may pair with the distal heterochromatin in two ways which may be described as eucentric and dyscentric. In those cases in which the ends of the two heterochromatic segments nearest the centromere are paired and the ends most remote from the centromere are paired the pairing is eucentric (see figure 2). Where the basal end of one segment pairs with the more remote end of the other segment, the pairing is dyscentric (see figure 3). These terms are used by DARLINGTON (1937)

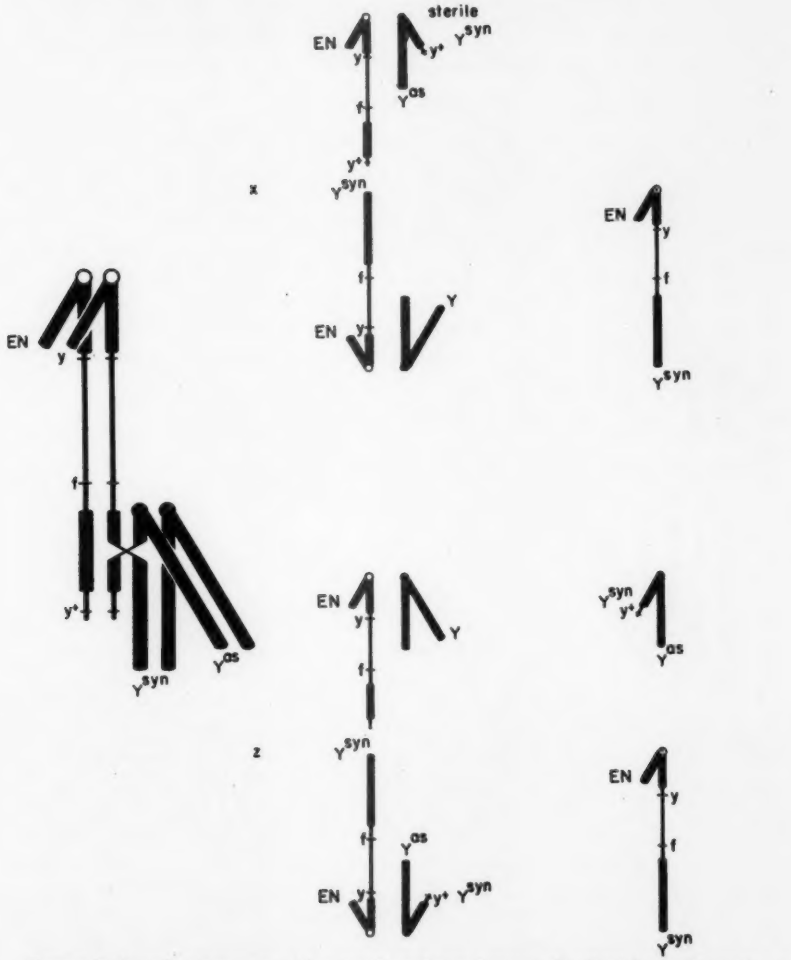

FIGURE 2. Genetic consequences of eucentric exchange between the distal heterochromatin of $In(1)sc^{sL},EN^{R}$ and the Y chromosome.

in a somewhat different connotation to describe gene arrangements in structural heterozygotes. Eucentric and dyscentric exchange will refer to ordinary exchange following these types of pairing.

Following a somatic exchange there are three possible types of two by two segrega-

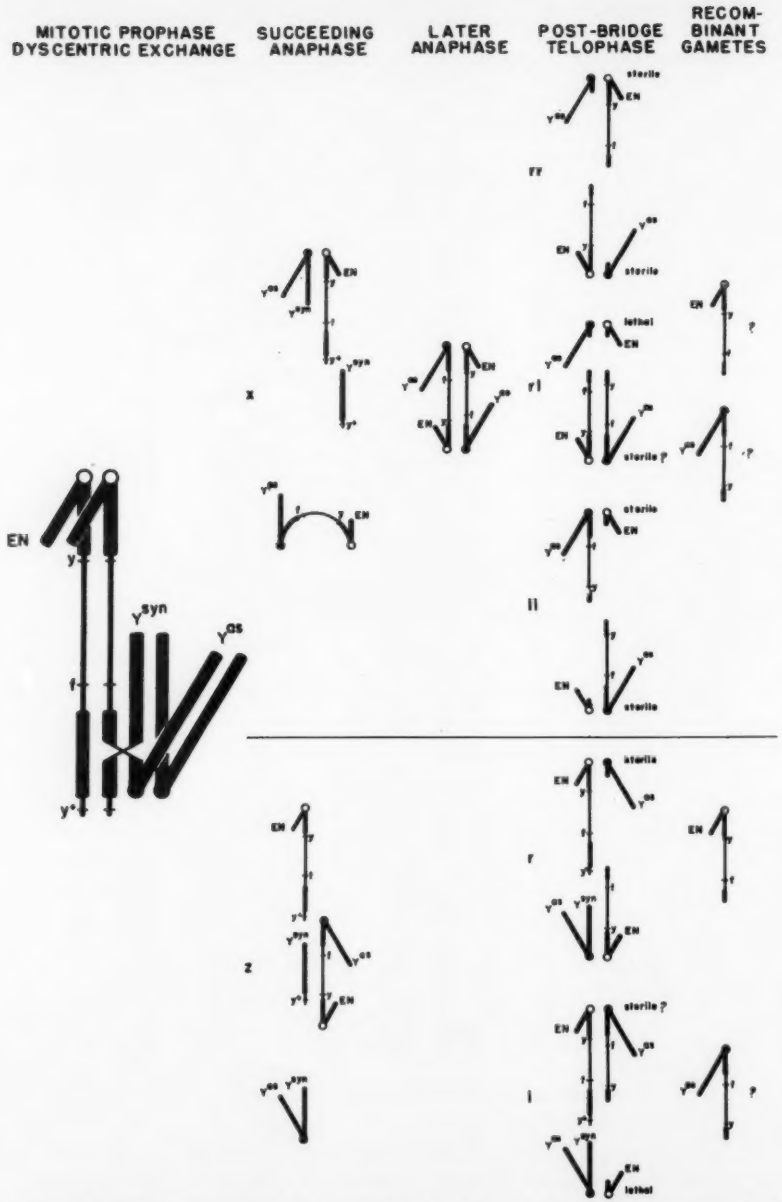

FIGURE 3. Genetic consequences of dyscentric exchange between the distal heterochromatin of $In(1)sc^{8L}$,EN^{R} and the Y chromosome.

tion from the tetrad (STERN 1936). The chromatids may separate equationally with one recombinant and one non-recombinant proceeding to each pole or the two recombinants may proceed to one pole and the non-recombinants to the other. These two types of segregation have been termed the x and z types respectively by Stern, and they will be so termed below. The third type of segregation, y, is reductional, and according to STERN does not occur. Henceforth only x and z segregation will be considered.

Eucentric pairing

Consider first the case of exchange proximal to all the fertility factors of Y^{syn}. This results in a recombinant X and Y. When x type of segregation occurs the recombinant X and the normal Y go to one pole; the resulting cell should give rise to a viable clone of cells even though, when the recombinant X has been derived from In(1)sc^8 (Experiments 3 and 4), such a cell is deficient for the *y-ac* region. Since *y-ac* deficient tissue is not cell lethal in the hypodermis (DEMEREC and HOOVER 1936; EPHRUSSI 1934), it is reasonable to assume that spermatogonial cells carrying deficient sc^8 c.o. X chromosomes should be able to proliferate. Such cells, however, may be rather inefficient in competition with normal cells, or a critical mass of deficient tissue may limit the size of clusters obtainable. To the other pole following type x segregation proceed the normal X and the recombinant Y; this cell should also give rise to a viable clone of cells, but since these cells lack fertility factors of the synaptic arm of the Y, whichever it may be, it is probable that they would be sterile. STERN and HADORN (1938) have established by transplantation techniques that male tissue deficient for Y^L, occurring contiguously with normal tissue in a mixed testis is unable to produce viable sperm as detected by progeny tests. From these results it can be said with fair certainty that if Y^L is the synaptic arm, the recombinant Y chromosomes resulting from eucentric exchange will not be found in functional sperm if x type segregation ensues. There must be some point, however, in the lineage of a spermatozoon between the germinal primordia and the secondary spermatocyte where the Y becomes unnecessary for completion of formation of functional gametes. If this point is prior to the first meiotic division the possibility of obtaining Y deficient primary spermatocytes which will finish normal development still exists. It may be reasonable to assume that the same facts outlined above with respect to Y^L apply equally well to Y^S, but experimental evidence is lacking.

Eucentric exchange and subsequent z segregation result in the passing of the two non-recombinant chromatids to one pole and the recombinant chromatids to the other; the cell receiving the non-recombinants is of no further interest. The other cell still has a complete chromosomal complement and should therefore have no difficulty in giving rise to a clone of cells which should yield equal numbers of recombinant X-bearing and recombinant Y-bearing gametes. Assuming x and z segregation to occur with equal frequencies and Y-deficient cells to be invariably sterile, a ratio of 2 recombinant X's to 1 recombinant Y is expected following eucentric exchange, at least from the In(1)sc^{sL},EN^R experiments. In the case of recombinants derived from eucentric exchange involving In(1)sc^8 this ratio may be modified slightly toward 1:1 due to the possible selective disadvantage of the *y-ac* deficient cells resulting from segregation of type x.

How are the above expectations modified by the occurrence of eucentric exchange distal to some or all of the Y^{syn} fertility factors? The resulting recombinant X chromosomes would not carry all of the fertility factors of Y^{syn} and, in the event that exchange was distal to all fertility factors, would carry none of them. Most if not all such recombinant X chromosomes would undoubtedly have gone undetected (by the method used) as having originated through exchange with the *Y* chromosome, since the test chromosomes (Y'' and Y^{cl}) probably confer fertility upon males only in the presence of all of the fertility factors of the complementary arm. The reciprocal recombinants, on the other hand, would carry all of the fertility factors of Y^{ss} plus some or all of those of Y^{syn}, and would have been invariably classified as recombinant Y's. This type of event would shift the ratio of recombinant X's to recombinant Y's in favor of an excess of recombinant Y's. This would be accomplished by reducing the number of detectable recombinant X's and possibly, in some cases, by increasing the likelihood that the recombinant Y-bearing cell derived from type x segregation would give rise to a clone of fertile cells.

The latter type of exchange leads to several experimentally testable expectations. First, if exchange were to occur distal to all of the fertility factors of Y^{syn}, the recombinant Y should carry a complete complement of fertility factors. Of seven tested recombinant Y's all lacked at least some Y fertility factors; these observations are corroborated in at least three additional cases by SIDOROV (1941) and CREW and LAMY (1940). MULLER, however, has recorded a recombinant Y from $In(1)sc^8$ which carries a full complement of fertility factors ($sc^8 \cdot Y$, MULLER 1948). Secondly, if the region of the fertility factors on Y^{syn} is pictured as one with several loci between which exchange may occur, any recombinant X resulting from exchange within this region should be fertile in combination with a recombinant Y arising through exchange at any point distal to that giving rise to the recombinant X, although the recombinant X need not be fertile in combination with Y'' or Y^{cl}. Consequently, roughly half of the combinations of recombinant X/recombinant Y, in which both recombinants are derived from exchange within the fertility factor region, should be fertile. From 38 recombinant X's and 7 recombinant Y's, 227 of the 266 possible combinations were tested for fertility. No case of a fertile combination in which the recombinant X was not also fertile with Y^{cl} was recorded.

Dyscentric pairing

It is seen (figure 3) that dyscentric exchange results in the formation of a dicentric and an acentric, with Y^{syn} fertility factors and y^+ being lost as an acentric in the case of exchange proximal to some or all of Y^{syn} fertility factors. Segregation of type x sends non-recombinants to one pole and the intact dicentric to the other. The non-recombinant cell need not be further considered, but the possible products of the dicentric are of interest. During the ensuing mitosis the dicentric reduplicates and sister centromeres separate, those at one end of the dicentric presumably separating independently of those at the other. This being the case, two bridges may be formed as shown in figure 3 or one dicentric may proceed, either freely or interlocked with its sister, to either pole. Each dicentric that survives a given mitotic dividion intact is again subjected to the possibility of bridge formation in the succeeding division. Type z

segregation on the other hand results in the immediate formation of a bridge and the passage of the non-recombinant X and Y to opposite poles.

The subsequent fate of the dicentrics once they have formed bridges must now be considered. Some recombinant X's recovered from the experiments reported in this paper are reinverted; this fact leads to the supposition that mitotic bridges may break giving rise to fragments which are able in subsequent mitotic divisions to behave as normal chromosomal elements. Precedent for such a notion is found in the work of STERN (1936) who has described hypodermal spots observed in ring/normal and inversion/normal heterozygotes whose explanation demanded rupture of a dicentric chromosome formed through somatic crossing over. It is practical to consider only breaks occurring in heterochromatin as is done in figure 3, since cells carrying fragments resulting from euchromatic breakage, if they were viable and, what is less likely, were able to develop into functional gametes, would in the vast majority of cases fail to give rise to viable zygotes.

As pointed out above, segregation of type x following dyscentric exchange results in the formation of two bridges in one or more of the succeeding mitoses; these may both break to the left of *y* (ll) or to the right of *f* (rr), or one may break to the right of *f* while the other breaks to the left of *y* (rl). Examination of Figure 3 reveals that rr or ll breakage arrangements result only in cells with incomplete Y's, which probably, therefore, never yield functional gametes; breakage of interlocked dicentrics yields cells similarly Y deficient. Bridge breakage of the rl type also results in a cell lacking a complete Y chromosome, but this cell is of the constitution 2X:2A. Two questions may be asked about a cell of this nature: Do patches of genetically female cells arising premeiotically in a testis complete spermatogenesis? If so, do such cells require all or part of a Y chromosome for fertility? The answer to the first question is affirmative; NEUHAUS (1937) has reported clusters of attached X females among the progeny of males carrying $X \cdot Y^S$; these attached X's are presumably products of eucentric exchange between the Y^S arm of one chromatid of the $X \cdot Y^S$ and the basal heterochromatin of its sister. Disjunction should occur such that an attached X passes to one pole, and a $Y^S \cdot Y^S$ chromosome passes to the other; the free Y chromosome should undergo normal mitotic division sending a chromatid to each pole. In some of his experiments NEUHAUS used $X \cdot Y^S/Y$ males and in others he used $X \cdot Y^S/Y^L$ males, but he fails to specify whether clusters of attached X's were recovered from one or both types. This point is important with respect to the second question raised above; if any such clusters were recorded from $X \cdot Y^S/Y^L$ males, the unimportance of Y^S in conferring fertility upon 2X:2A spermatogonial cells would be indicated. Type z segregation followed by r breakage of the bridge thus formed yields one cell lacking Y^{syn} and another with a non-recombinant Y plus an X derived from bridge breakage; this cell should be able to give rise to recombinant X-bearing gametes. l breakage yields a 2X:2A cell subject to the same questions as similar cells arising from x segregation.

Examination of figure 3 reveals that only one type of recombinant would be recovered with certainty following the types of events described above; this is the X fragment resulting from z segregation followed by r breakage. Such a fragment would carry no Y arm and hence would be indistinguishable from the many recombinants

recovered in which Y chromosome involvement could not be demonstrated. The recombinant types which would be recoverable if the 2X:2A cells in question gave rise to viable gametes are of two types. One is identical to that recovered from z segregation followed by r breakage, and the other is a reinverted chromosome carrying a complete Y arm proximally as a second arm. All of the recombinant X's were tested for sequence by crossover tests with normal and In(1)sc^{s} sequences; all of those which carry Y^{s} were found to have retained their inverted sequence and to carry the Y^{s} fertility factors distally. Also none were found cytologically to carry a second arm.

As in the case of eucentric exchange, the question of exchange distal to Y fertility factors is important in a treatment of dyscentric exchange. The foregoing discussion has considered exchange proximal in Y^{syn} to some or all of the fertility factors; how is this discussion modified in the case of exchange distal to all fertility factors? Such exchange would result in the inclusion of all of the fertility factors of Y^{syn} in the bridge; subsequent l breakage would give rise to a reinverted X with a complete Y chromosome proximally located. Viable and fertile cells containing such an X would be produced by x segregation followed by ll breakage; also 2X:2A cells carrying such an X would result from z segregation followed by r breakage. No such recombinant has been found, however. Breakage at the right end of a bridge containing the fertility factors of Y^{syn} would yield different results depending upon which side of the fertility factors the break were to occur. Fragmentation to the right of all of the fertility factors would yield an X with Y^{syn} carried distally; such a fragment would occur in 1X:2A cells with a full Y complement following x segregation and rr breakage and following z segregation and r breakage, or in 2X:2A cells with a full Y complement following x segregation followed by rl breakage. Fragmentation to the left of the fertility factors would not yield any new types of recombinants, but it would confer fertility upon the cell bearing an X fragment with no Y arm resulting from x segregation followed by rr breakage. It is evident that dyscentric exchange distal to the fertility factors of Y^{s} gives rise to a bridge which, if broken at the right and to the right of the included fertility factors, yields a product which is indistinguishable from recombinant X's postulated as coming from eucentric exchange proximal to the fertility factors of Y^{syn}. When the break is to the left of the fertility factors, however, a product indistinguishable from those recombinant X's not demonstrably involving the Y results. Breakage at the left end of such a bridge yields a product not yet recovered. Since there is no a priori reason for expecting breakage of such a bridge to occur preferentially at one end, the absence of the latter type of product can probably be taken as an indication that few if any of the products recovered arose following such a dyscentric exchange.

It should be profitable to review now the various recombinants which have involved the Y chromosome in view of the foregoing discussion. All of the recombinant Y's must have originated through eucentric exchange, since they carry the plus allele of *y* which is invariably lost as an acentric following dyscentric exchange. Of seven recombinant Y's tested (three from In(1)sc^{sL},EN^{R} males, two from In(1)sc^{s} males and two from In(1)sc^{sl} males (MULLER-5)), all lacked Y^{s} fertility factors while carrying all of those normally found on Y^{L}; in all seven cases, therefore, the short arm of the

Y was the synaptic arm. It seems likely that in every case exchange was proximal to all of the fertility factors since each was sterile in all combinations tested with 27 recombinant X's which were known to be sterile with Y^{cl}.

The interpretation of the recombinant X's is more complicated; of thirty-four recovered from $In(1)sc^{sL},EN^{R}$ males four could be demonstrated to carry all of the fertility factors of Y^{S} as could six out of ten recombinant X's recovered from $In(1)sc^{s}$ males; none however could be demonstrated to carry Y^{L} fertility factors. Since each of the ten recombinant X's carrying Y^{S} still retains its original inverted sequence and carries Y^{S} distally, all must have originated through eucentric exchange proximal to the fertility factors or dyscentric exchange distal to the fertility factors; in either event, however, Y^{S} must have been the synaptic arm. As stated previously, dyscentric exchange distal to Y^{syn} fertility factors produces a bridge within which lie all the Y^{syn} fertility factors, and since there is no reason to believe that breakage of a bridge should occur preferentially at one end or the other, the absence of the one distinctive bridge breakage product among the recombinants studied probably indicates that breakage of this particular type of bridge contributes little if at all to the recombinants recovered. Consequently the recombinant X's which carry Y^{S} must have arisen preponderantly through eucentric exchange. One of these recombinant X's however, may have arisen through such dyscentric exchange. sc^{s} c.o. X's 67h(1) and 67h(2) were recovered from the same father; 67h(1) is inverted and does not carry all fertility factors of either arm, and 67h(2) is inverted and carries all fertility factors of Y^{S} distally. Although these recombinants are readily explainable by postulating two events, if possible the occurrence of two recombinants from a single male should be explained as the consequence of a single event rather than two independent and rather rare events. The only scheme (see figure 3) that seems to account for all of the observations is as follows: (1) Dyscentric exchange between sc^{s} heterochromatin and Y^{S} distal to fertility factors followed by x segregation. (2) Separation of two intact dicentrics for at least one subsequent mitosis. (3) Formation of two cells which produce double bridges at anaphase. (a) rr breakage of the bridges of one cell to the right of the fertility factors such that each daughter cell receives Y^{L} from one bridge and an inverted X with Y^{S} fertility factors distally located (67h(1)) from the other; and (b) rr breakage of the bridges of another cell to the left of the fertility factors such that each daughter cell receives a recombinant X lacking Y^{S} fertility factors (67(2)) from one bridge and a complete Y from the other.

A second cluster of interest is E_1; this cluster was recovered from experiment 3 and consisted of two *y* females which carried recombinant X's with Y^{S} located distally, and one *B* male which was presumed to be of the constitution $Y^{S}X \cdot Y^{L}$, In(1)EN, *B* y/sc^{s} c.o. Y. This male was crossed to attached X females; the F_1 consisted of matroclinous females and *B* males. The y^{+} allele which was presumed to have originated from the paternal $In(1)sc^{s}$ chromosome appeared to be segregating with the maternal $Y^{S}X \cdot Y^{L}$ chromosome. The possibility of reverse mutation of *y* in $Y^{S}X \cdot Y^{L}$ was considered, but further analysis showed that this chromosome is the $Y^{S}X \cdot Y^{L}$, In(1)EN, *B y* chromosome, but with the terminal Y^{S} replaced by the y^{+} from $In(1)sc^{s}$. The following events may be postulated to account for this unusual combination of maternal and paternal chromosome elements: The original zygote

received a sc^8 c.o. Y from its father and a $Y^8X \cdot Y^L$, In(1)EN, *B y* chromosome from its mother; in one of the early cleavage divisions eucentric exchange between the Y^8 arm of the sc^8 c.o. Y and the distal Y^8 of the $Y^8X \cdot Y^L$ chromosome occurred. Subsequent segregation of type z resulted in daughter cells of the composition $X \cdot Y^L$, $In(1)sc^{8L},EN^R$, y^+ *B* y/Y and $Y^8X \cdot Y^L$, In(1)EN, *B* y/sc^8 c.o. Y. Only the former type gave rise to germinal primordia, whereas both types could have participated in hypodermis formation, since they both are y^+ *B* in phenotype. That such an exchange can occur in early cleavage is testified to by the fact that a patroclinous son has been recovered from an $In(1)sc^{8L},EN^R$ male which was bilaterally mosaic for y and y^+; this male when crossed to attached X females produced only recombinant X sons.

Finally it should be reemphasized that the only product of bridge breakage following dyscentric exchange proximal to Y^{syn} fertility factors which one would expect with certainty to give rise to functional gametes is one which cannot be demonstrated to have involved the Y. It is therefore not possible to properly assess the role of dyscentric exchange between X and Y in the formation of recombinant X's. Some proportion of the recombinant X's which cannot be demonstrated to have involved the Y may actually be products of dyscentric exchange.

SPECIFICITY OF Y^8

The present paper considers sixteen cases of recombination between the distal heterochromatin of $In(1)sc^8$ or $In(1)sc^{81}$ and the Y chromosome. Each of these cases has involved the short arm of the Y. To this list can be added six recombinant X's and at least two recombinant Y's reported by SIDOROV (1941) and the recombinant Y reported by CREW and LAMY (1940), or a total of 24 cases of exchange involving the short arm of the Y. The only recorded exception to the generalization that Y^8 is the synaptic arm is the case of $sc^8 \cdot Y$ of MULLER (1948); here the exchange seems to have involved Y^L distal to the fertility factors. Exchange giving rise to heterochromatic recombinants is probably a function of mitotic pairing of the regions involved, and it may be that the preferential involvement of Y^8 in such exchange provides a measure of competitive pairing abilities of Y^8 and Y^L in the situation studied; this homology indicated by pairing need not, however, be correlated with the degree of genetic homology between a given Y arm and the X heterochromatin.

HOMOLOGOUS VERSUS NON HOMOLOGOUS EXCHANGE

As mentioned earlier, exchange between the distal heterochromatic region of $In(1)sc^8$ and Y^8 in such a way that two monocentric recombinants are formed suggests non-homologous pairing. Such a suggestion, however, presupposes some knowledge of homologous pairing, but, as will be pointed out in the discussion, little is known of the nature of the homologous region shared by X and Y.

Let us investigate, however, the possibility of differentiating between the alternatives of strictly homologous exchange and indiscriminant non-homologous exchange, without assuming anything as to the nature or extent of the homologies between X heterochromatin and Y^8. With respect to the first possibility, regardless of how these homologous regions are split up and rearranged, there will exist regions of

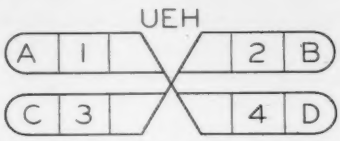

FIGURE 4. Schematic representation of the consequences of exchange between regions of uninterrupted eucentric homology (UEH).

uninterrupted homology; the size and number of these regions will depend on the complexities of the sequential differences between the X heterochromatin and Y^S A region of uninterrupted homology in the X may be inverted or not with respect to its counterpart in Y^S so that homologous pairing may be dyscentric or eucentric. For convenience, let us confine our attention to regions of uninterrupted eucentric homology (= UEH regions). It should be pointed out that a region which is not a UEH region in a normal male may become so in a sc^8 male, because it is reversed by the inversion.

Figure 4 is a generalized diagram of the outcome of exchange within UEH regions. A, B, C, and D represent regions completely outside of the region of homology, for example the euchromatin (A) and the centromere (B) of a normal X chromosome (*y-ac* (A) and the rest of the euchromatin and the centromere (B) of $In(1)sc^8$) and the Y^S specific fertility factors (C) and the Y centromere and Y^L (D). Regions 1, 2, 3, and 4, on the other hand, represent portions of the homologous regions outside the particular UEH region in question. Whereas A, B, C, and D are constant, the extent of 1, 2, 3, and 4, must differ with each different UEH region. Examination of the figure reveals that any exchange within the UEH region yields two and only two recombinant types of constitution A 1 UEH 4 D and C 3 UEH 2 B (*i.e.*, only one recombinant X and one recombinant Y). Said in another way, one can demonstrate as many different UEH regions as he can demonstrate different classes of recombinant Y's, or, similarly, recombinant X's. If the number of classes of recombinants indicates that the number of UEH regions is large, the notion of strictly homologous pairing becomes less convincing than that of non-homologous pairing, especially since this region is represented by 8–10 polytene chromosome bands (PROKOFYEVA-BELGOVSKAYA 1937). It might be mentioned that the same argument applies to the dicentric chromosomes formed by dyscentric exchange between X and Y, but breakage at different levels of the bridges subsequently formed would obscure any regularities in classes of dicentric chromosomes. The generalization outlined above holds strictly only for a particular X and a particular Y; the possibility should be pointed out, however, that different Y's or for that matter the heterochromatin of different sc^8 stocks may differ sequentially. Such a complication could result in variations in the constitution of regions 1, 2, 3 and 4 from chromosome to chromosome with respect to the same UEH region; consequently recombinant X's or recombinant Y's derived from exchange within the same UEH region in chromosomes of different origin need not in the final analysis be identical.

In view of the relationships discussed in the preceding paragraphs, a thorough examination of recombinants between X and Y^S was undertaken with the hope of

being able to subdivide them into several classes. Since the homologous regions of the X and Y chromosomes used were not known to differ with respect to any markers, duplications and deficiencies for known markers were the only possible criteria. Although little is known of the homologies between X and Y, the known order of heterochromatic factors in the X chromosome permits predictions to be made as to the most likely types of duplications and deficiencies to be observed among the recombinants studied. A tentative order of useful landmarks in the X heterochromatin is, from left to right in a normal sequence, bb^+, nucleolus organizer (*NO*) (see KAUFMANN 1944), block A, block B (as defined by MULLER *et al.* 1937), the right breaks of $In(1)sc^8$ and $In(1)sc^{81}$, block B (as defined by GERSHENSON, 1940). Since methods of detecting block B are poorly defined it will henceforth be omitted from the discussion. The order of factors in the distal heterochromatin of $In(1)sc^8$ is block A most distally, nucleolus organizer, and bb^+ most proximally. If in figure 4 A represents the *y ac* region of $In(1)sc^8$ and B the portion of $In(1)sc^8$ proximal to the distal heterochromatic segment, for any UEH region the known heterochromatic factor most likely to lie in region 1 is block A and the most likely factor to be found in region 2 is bb^+. The factor which any recombinant Y is least likely to have picked up from the X, therefore, is bb^+, while the factor that recombinant X's are most likely to have lost is block A. These losses, of course, may well have been compensated for by the contribution of the Y to a particular recombinant. Each recombinant Y was combined with $In(1)sc^{4L},sc^{8R}$, a chromosome deficient for the region including *bb NO bkA*. Six of seven cases studied gave rise to viable but sterile males; the seventh combination (B-2) was lethal. Recombinant B-2 in combination with *bb* gives flies which are phenotypically bobbed and in combination with bb^l (= bobbed lethal) is lethal. With the reservation mentioned above with respect to sequentially different X's or Y's, these two types of recombinant Y's define two different UEH regions; that defined by the first six is located such that at least one *bb* locus is contained in region 1, UEH, or 4, whereas that defined by the bobbed deficient recombinant Y is located such that the *bb* loci of X and Y are located in regions 2 and 3 respectively. Of eleven recombinant X's carrying Y^S, nine were tested as XO males and found to be viable and normal in phenotype; they were, therefore, not deficient for any genetically demonstrable heterochromatic factors. Those derived from $In(1)sc^{8L},EN^R$ had to have the unknown heterochromatic right end replaced by that of $In(1)sc^8$ before the above observations could be meaningful; furthermore the XO males had to carry $Dp(1;3)sc^{J4}$ to eliminate inviability caused by deficiency for *y-ac*.

The presence of block A is not subject to genetic test since, as defined by MULLER *et al.* (1937), it is necessary for neither viability nor fertility. A cytological examination of all of the chromosomes discussed above was undertaken to observe the distribution of block A among the recombinants. The material chosen was prophase of larval neuroblasts, since the contrast between euchromatin and heterochromatin is so marked at this stage. A normal X chromosome consists of an attenuated euchromatic portion of variable length, depending upon how far prophase has advanced, and of three terminal heterochromatic segments separated by two constrictions. The first segment is extremely small and is separated from the other two by the primary constriction; this segment is the short arm of the X and is undetectable in

many cells. The other two segments are approximately equal in size and are slightly larger than the fourth chromosomes. They are separated by the nucleolar constriction which in early prophase is often a gap of considerable size. The right break of In(1)sc^{8} is in the more proximal of the two large segments so close to the primary constriction as to leave only a minute portion of this segment proximally; the right break of In(1)sc^{L8}, on the other hand, is in the same segment but so close to the nucleolar constriction as to remove only a minute portion of this segment distally (COOPER—personal communication). It is the region between these two breaks that has been defined as block A (plus block B) by MULLER *et al.* (1937). All eleven recombinant X's carrying Y^{S} are indistinguishable from In(1)sc^{8}; *i. e.*, they carry distally two large heterochromatic segments which do not differ appreciably in size. Even in early prophase the Y chromosome is quite fully condensed, and often not bent at the primary constriction. This makes it rather refractory for cytological study; none of the seven recombinant Y's, however, was found to differ appreciably in morphology from a normal Y chromosome. These observations, then, fail to further subdivide the classes of recombinant X's or Y's.

The recombinant Y recovered by CREW and LAMY (1940) from In(1)sc^{81} has been reported by them to be a rod shaped chromosome. PONTECORVO, however, describing the same chromosome later (1940) reported that it is a V-shaped chromosome with one arm being Y^{L} and the other being equal to or a little greater in length than a normal X. In either event the above case is in a different class from the two classes of recombinant Y's discussed above, both of which resemble a normal Y cytologically.

The results of NEUHAUS (1937—see discussion) concerning exchange between the heterochromatin of a normal X and Y^{S} demonstrate a UEH region which includes the locus of *bb* or two UEH regions, one on either side of the *bb* locus, but proximal to the fertility factors of Y^{S}. Since a region which is UEH in a normal sequence cannot by definition be UEH in inverted sequence, NEUHAUS' results demonstrate a fourth region of uninterrupted homology. Furthermore, if the explanation proposed above for cluster 67h is correct a fifth region of uninterrupted homology distal to the fertility factors of Y^{S} and distal to *bb* *NO* and probably *bkA* of In(1)sc^{8} is indicated. The seriousness of the ambiguity posed by the fact that these five regions of uninterrupted homology have been inferred from a consideration of recombinants from more than five different crosses in which Y's of different origin have been used is difficult to assess.

DISCUSSION

Evidence in favor of homology between the X and Y chromosomes is forthcoming from many kinds of observations. The homologies indicated are of two types, genetic and pairing. Genetic homologies, *i.e.*, the sharing of similar loci, are primarily demonstrated by genetic methods. Strictly speaking, pairing homologies can be demonstrated only cytologically, but presumably they are correlated with disjunctional behavior and exchange behavior. The extent of the correlation between genetic homology and pairing homology is of interest.

Evidence favoring genetic homology between X and Y is found in the demonstra-

tion of similar genetic factors in both chromosomes. As shown by STERN (1927) both the X and the Y chromosomes carry the locus for bobbed. From a cross of *y bb*/Y males to attached X females carrying the short arm of the Y (Y^S), NEUHAUS (1937) checked 26,557 F_1 males for fertility; among these six fertile males were found which carried $X \cdot Y^L$. These chromosomes had presumably arisen through exchange between X heterochromatin and Y^S, and should, therefore, have carried all of the long arm of the Y. Four of the six carried bb^+ and two carried *bb*; this indicates that Y^S must carry bb^+. An ambiguity, but probably not a serious one, is introduced by the possible origin of $X \cdot Y^L$ through exchange between Y^L and the right arm of the X. In earlier crosses NEUHAUS (1936) had obtained from homozygous $X \cdot Y^L$ females rod shaped Y^L chromosomes; from the fact that males carrying bb^l/Y^L were extreme *bb* in phenotype he concluded that Y^L carries an extreme allele of *bb*. The latter observations are puzzling for a number of reasons and his conclusions may not be valid. An indication of the latter point is the fact that MULLER's Y^{cl}, which almost certainly carries all of Y^L from the centromere to the fertility factors inclusively and at least part of Y^S is lethal in combination with bb^l and not viable as would be expected if Y^{cl} carries a *bb* locus.

BESMERTNAIA (1934) working with DUBININ noticed that some translocations involving the Y chromosome and chromosome II or III, which were fertile in normal males, were sterile or partially sterile in bb^{def} males. It is possible that these observations indicate allelism of certain fertility factors on X and Y; they may, however, indicate some phenomenon of more general significance. Among eight Y; 4 translocations which were fertile in normal males, NEUHAUS (1939) found one to be sterile in bb^{def} males. This translocation could be shown to involve the long arm of the Y.

One further indication of the homology between the Y chromosome and the heterochromatin of the X is the cytological demonstration of the presence of the nucleolus organizing region at the base of the X and in the short arm of the Y chromosome (HEITZ 1933).

Although ample evidence exists favoring genetic homology between X and Y, practically nothing can be said with respect to the extent or the arrangement of the homologous regions. A fair amount of information is available concerning the linear distribution of the known heterochromatic factors in the X chromosome, but comparable data for the Y are by and large non-existent. NEUHAUS (1939) has, on the basis of the fertility of males carrying different combinations of Y; 4 translocations, designated ten fertility factors, five located distally on Y^L and five located distally on Y^S. He has provisionally placed one of the alleles of *bb* immediately proximal to the proximal most fertility factor of each arm. His reasons for doing so, however, have not been clearly stated.

The normal disjunction of X from Y in males and the abnormal disjunction of X from X in XXY females (secondary non-disjunction, BRIDGES 1916) demonstrate genetically a pairing relationship between X and Y. That the heterochromatic region of the X is involved in this relationship was demonstrated by MULLER and PAINTER (1932) who showed that the right portion of an X involved in a T(1;4) with one break in the region of *f* segregates fairly regularly from the Y chromosome, whereas the left portion segregates essentially at random with respect to the Y. Furthermore it

has been shown that X-Y disjunction in males carrying an X chromosome lacking a major portion of the heterochromatic region (*i.e.*, In(1)sc^{4L},sc^{8R}) is distinctly abnormal (GERSHENSON 1934; SANDLER and BRAVER 1954). These observations receive cytological corroboration in the studies of spermatogenesis by COOPER (1949) who observed 100% X-Y associations at first meiotic metaphase; the two chromosomes were associated over but a small proportion of their length, conjugation being preponderantly interstitial in the X and proximal in the Y. That these associations may not, however be strictly comparable to homologous pairing, in the generally accepted sense of the word is testified to by the fact that the entire sex chromosome complement is always associated into a multivalent in XYY and XYYY males. COOPER (1951, and unpublished) has furthermore stated that associations between X and Y involve specific heterochromatic regions (collochores) and are such that any collochore on the X may become associated with any collochore on the Y, or, in the case of an inverted X in which the right break is in the heterochromatin, a distally located collochore on the X may become associated with a proximally located collochore on the same X. From studies of the rates of secondary non-disjunction in males and females hyperploid for various portions of the heterochromatic regions of the X chromosome present as free fragments, GERSHENSON (1940) has inferred an array of pairing loci similar in distribution to COOPER's collochores.

In studies of polytene chromosomes, PROKOFYEVA-BELGOVSKAYA (1937) has observed strict band for band pairing between X and Y over the entire heterochromatic region of the X, a section of eight to ten bands in the chromocenter. No indication of the two armed nature of the Y chromosome was demonstrable; in fact configurations involving Y fragments which were presumed to carry a single arm were indistinguishable from those involving intact Y chromosomes.

Studies of exchange between X and Y, with respect to homologies indicated, have generally been fraught with ambiguities; some of these difficulties were mentioned briefly in the introduction to this paper and others have been treated in detail throughout the preceding pages. Cases of exchange between a normal X and a normal Y have been recorded; the first was probably XY′ reported by STERN (1929). This is an X chromosome with Y^{L} appended as a second arm; it was derived from a *bb*/Y male and is itself *bb*$^{+}$ in phenotype. Similar cases of recovery of X·Y^{L} chromosomes from normal males have been recorded by NEUHAUS (1937—see above). In these cases, as in those reported in the present work Y^{S} was the synaptic arm, but not necessarily to the exclusion of Y^{L} since the crosses employed by NEUHAUS were such that X·Y^{S} would not have been recovered. Conclusions drawn from recombinants derived from a two-armed X should be viewed with caution for two reasons. First, studies of exchange between two-armed X's (*e.g.*, Dp(1;1)Theta—STERN and DOAN 1936, and attached X's—KAUFMANN 1933 and NEUHAUS 1935 and 1936) and the Y may theoretically involve either arm of each chromosome; if for instance the short arm of Theta were to undergo exchange with the short arm of the Y the resulting recombinants would be indistinguishable from those arising by exchange between the long arm of Theta and the long arm of the Y. The above investigators have, of course, recognized this difficulty. The extent to which the right arm of the normal X poses the same difficulty is probably slight. A second and less generally

recognized difficulty with two armed X's is that they almost certainly carry the Y centromere and at least the proximal regions of each Y arm. This situation has been clearly demonstrated by MRS. MORGAN (1938) in her descriptions of the origin of several independently recovered attached X chromosomes. A difference between the centromere regions of one-armed and two-armed X's has also been demonstrated by NOVITSKI (1952) with respect to their behavior in double first anaphase bridges. It is possible, therefore, that some cases of exchange between a two-armed X and a Y involve regions of Y origin exclusively, regions, furthermore, which may not undergo exchange with the X. Products of such an exchange would not differ detectably from recombinants arising by X-Y exchange. One can imagine also regions shared by Y^S and Y^L which can undergo exchange. If such regions exist data on exchange between the arms of a two-armed X (see NEUHAUS 1936, 1937) are subject to the same ambiguity as that mentioned above with respect to exchange between a two-armed X and the Y.

The experiments described in this paper definitely seem to demonstrate preferential participation of the short arm of the Y chromosome in exchange with the distal heterochromatin of In(1)sc^8. It is furthermore suggested that such exchange might not necessarily involve strictly homologous regions of the chromosomes. This contention is not considered to have been demonstrated indisputably, but it is felt that a proper experimental approach to the question has been presented. In view of COOPER's observations on specific but apparently non-homologous associations between collochores, an attractive hypothesis is that heterochromatic regions in general form specific but non-homologous associations (*e.g.*, chromocenter formation) and, given the proper conditions, may subsequently undergo non-homologous exchange. A corollary to this hypothesis might be that what has been termed above pairing homology should better be thought of as a non-homologous pairing specificity when heterochromatin is involved, and it might follow that disjunctional behavior, insofar as it is conditioned by heterochromatic regions, is more closely correlated with these pairing specificities than it is with genetic homology. Heterochromatic exchange is not known to differ qualitatively between males and females or between meiotic and mitotic divisions; consequently the above hypothesis may be considered to apply equally well to all cases. It may be that in the future information will become available which will require certain qualifications to be made with respect to the general nature of the hypothesis proposed above.

SUMMARY

Spermatogonial exchange may occur between the heterochromatic segment of the X chromosome shifted distally by In(1)sc^8 and the Y chromosome. This exchange may occur in such a way that two monocentric recombinants result; one is a Y chromosome carrying the euchromatic region distal to the distal heterochromatin of In(1)sc^8 (y^+ and ac^+) in place of the distal portion of one of its arms. This arm was the short arm in seven cases studied. The other recombinant is an X chromosome lacking y^+ and ac^+ but carrying distally the distal portion of one of the arms of the Y. This has been Y^S in ten cases tested. It is possible that exchange may also occur such that one dicentric and one acentric recombinant are formed.

It is shown that for a given X and Y, if strictly homologous pairing and exchange occur, the number of different types of recombinants recovered provides a direct measure of the number of differences in sequence between the two heterochromatic segments. If this number becomes large, on the basis of the knowledge that the region involved is of no more than eight to ten salivary chromosome bands in length, the case for strictly homologous exchange becomes less convincing than that for non-homologous exchange. At most five such segments have been demonstrated, and on the basis of this finding the hypothesis has tentatively been advanced that pairing and subsequent exchange and/or disjunction involving heterochromatic regions are conditioned by non-homologous but heterochromatin-specific forces.

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GENETIC VARIANCES IN OPEN POLLINATED VARIETIES OF CORN[1]*

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INVESTIGATIONS of quantitative inheritance in corn have been in progress at the North Carolina Experiment Station for the past seven years. While data have been collected on a number of characters, the primary objectives have been (a) measurement of the additive genetic variance in grain yield in corn populations and (b) estimation of the level of dominance involved in the action of genes affecting grain yield. Previous reports, ROBINSON *et al.* (1949) and GARDNER *et al.* (1953) have related to studies on hybrid populations derived from single crosses of inbred lines. They have been consistent and can be briefly summarized.

(a) Estimates of additive genetic variance have been large enough to indicate that selection, either single plant or among progenies, should be effective in improving the yield of such hybrid populations. Selection studies, designed for comparison of actual with predicted progress and to indicate the rate at which additive genetic variance is dissipated, are in progress.

(b) Estimates of the average dominance of yield genes have been in the overdominance range. However, it was recognized from the outset that both linkage and interaction of non-allelic genes were possible sources of upward bias in these estimates. Thus, strictly speaking, the results serve only to indicate an upper limit for dominance in yield inheritance and by themselves do not constitute final evidence for overdominance.

Complementary work with open-pollinated varieties was initiated in 1949. Early corn breeding experience had led to the widespread belief that selection for yield in adapted varieties is essentially ineffective. However, there is known to be considerable intra-variety genetic variation. Why should selection be ineffective? A possible explanation has been provided by HULL (1945) who postulates in effect that the genetic variance in such populations is largely non-additive and hence that progress through selection should not be expected. He points out that corn and corn varieties have been under selection for yield for years past. Assuming partial or complete dominance, the effect of continued selection on the population frequencies of a pair of alleles should be to cause the frequency of the more favorable, the plus, allele to approach ever closer to *one* until very little genetic variation of any sort remains. However, assuming overdominance the result is different. Because the heterozygote is favored, the effect of selection is toward holding both alleles in the population, rather than toward eliminating one of them; the population gene frequencies gravitate toward an equilibrium at intermediate values for both alleles.

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When this equilibrium is reached, selection has no further effect. Both alleles remain in the population, but, while it was not stated in these terms by HULL, the genetic variance produced by their segregation is dominance variance; at most only a trivial amount of additive genetic variance remains.

This explanation, for which supporting arguments have been advanced by CROW (1948), BRIEGER (1950), and some of the contributions to HETEROSIS (1952), immediately suggests that the nature of genetic variance in open-pollinated varieties be examined. Clearly, to find considerable dominance variance but only a negligible amount of additive genetic variance would constitute strong supporting evidence for the overdominance theory. Because of the extended opportunities for recombinations to bring linkage relations in varieties to equilibrium, no complications from linkages are to be expected. By equilibrium in the linkage relation between two loci is meant a state where the relative frequency of the four kind of gametes AB, Ab, aB, and ab is $q_A q_B$, $q_A(1 - q_B)$, $(1 - q_A)q_B$ and $(1 - q_A)(1 - q_B)$ as when there is independent segregation. q_A is the frequency of A and q_B the frequency of B.

MATERIALS AND METHODS

Biparental progenies were made within the Jarvis, Weekley and Indian Chief varieties as described for the study reported by ROBINSON *et al.* (1949). Each plant used as a male or staminate parent was crossed with four plants used as female or pistillate parents. Sixty-four such male groups or a total of 256 crosses were made within each of the Jarvis and Weekley varieties in 1949 which constituted the first of two samples of biparental matings made in these varieties. Four male groups, four progenies per male, were randomly chosen to form a block of 16 progenies and were tested with two replications, giving a block of 32 plots. A total of 16 such blocks of material were used within each of the two populations, making a total of 512 plots in each population. The same progenies tested in 1950 were tested in 1951, a new randomization within blocks being used the second year. The same type of biparental matings were made again in the Jarvis and Weekley varieties in 1951 to give a second sample of 256 crosses in each of these varieties. At the same time 512 such biparental crosses were made within the Indian Chief variety. These crosses were tested in 1952 and retested in 1953 with an arrangement of the progenies within each population similar to that described for the 1950 and 1951 tests. The studies were conducted in Wake County, North Carolina, and on the same farm in 1953 as in 1951 but on a different farm in each of the other two years. Plots were two rows wide and 10 hills long with three feet between rows and plants spaced 18 inches within rows.

Data were taken on date of flowering, plant height, ear height, kernel row number, number of ears, ear length, ear diameter and yield of ear corn in the Jarvis and Weekley populations. Five of the same characters were measured in the Indian Chief progenies, omitting kernel row number, ear length and ear diameter. Date of flowering was recorded by plots at the time when approximately 50% of the plants were shedding pollen. The data on all other characters were based on 10 competitive plants in each plot wherever possible. Plant and ear height were measured in inches

from ground level to the tip of the tassel and uppermost ear bearing node, respectively. Data on kernel row number, ear length and ear diameter were taken on only the topmost ear of the plant and the measurements taken to the nearest one-tenth inch. Data on number of ears and yield were based on all ears. Yield was measured on ear corn and recorded in pounds.

Plot values were obtained by dividing the total for a plot by the number of competitive plants that contributed to the total. Individual plant data (except in the case of date of flowering) on approximately every 12th plot were used for estimation of plant-to-plant variability within the plots.

Data for each of the characters of each sample of progenies in a population were analyzed by individual blocks and the results pooled to give the combined analysis for two years. The form of the analysis is given in table 1.

Under assumptions listed by COMSTOCK and ROBINSON (1952), the variance of

TABLE 1

Form of the analysis of variance computed for data for each sample of biparental progenies in three open-pollinated varieties of prolific corn

Source of variation	D. F.	M. S.	M. S. Expectations
Years	$t - 1$		
Blocks	$b - 1$		
Years x blocks	$(t - 1)(b - 1)$		
Reps in blocks in years	$bt(r - 1)$		
Males in blocks	$b(m - 1)$	M_1	$\sigma_w^2/k + \sigma_e^2 + r\sigma_{fy}^2 + rl\sigma_f^2 + rn\sigma_{my}^2 + rln\sigma_m^2$
Females in males in blocks	$bm(n - 1)$	M_2	$\sigma_w^2/k + \sigma_e^2 + r\sigma_{fy}^2 + rl\sigma_f^2$
Years x males in blocks	$b(m - 1)(t - 1)$	M_3	$\sigma_w^2/k + \sigma_e^2 + r\sigma_{fy}^2 + rn\sigma_{my}^2$
Years x females in males	$bm(n - 1)(t - 1)$	M_4	$\sigma_w^2/k + \sigma_e^2 + r\sigma_{fy}^2$
Pooled error	$bt(mn - 1)(r - 1)$	M_5	$\sigma_w^2/k + \sigma_e^2$
Total	$btmnr - 1$		
Plants in plots	$\sum_i (k_i' - 1)$	M_6	σ_w^2

KEY TO TABLE 1

t = years = 2
b = blocks per year = 16, except 32 for Indian Chief
r = replications per block = 2
m = male groups in a block = 4
n = females per male = 4
k_i' = number of plants in i^{th} plot on which individual plant data were taken
k = harmonic mean of the number of plants per plot
σ_w^2 = the sum of intra-plot environmental variance and the genetic variance among individuals of the same progeny
σ_e^2 = the variance of plot effects
σ_{fy}^2 = variance due to interaction between female effects and environment as it varied between years.
σ_{my}^2 = the interaction variance between male effects and environments
σ_f^2 = the variance of female effects
σ_m^2 = the variance of male effects

male effects (σ_m^2) equals one-fourth of the additive genetic variance (σ_g^2), and the variance of female effects (σ_f^2) equals one-fourth the additive genetic variance plus one-fourth the dominance variance (σ_d^2). Symbolically,

$$\sigma_m^2 = \tfrac{1}{4}\sigma_g^2$$
$$\sigma_f^2 = \tfrac{1}{4}\sigma_g^2 + \tfrac{1}{4}\sigma_d^2$$

so that $\sigma_g^2 = 4\sigma_m^2$. The dominance variance, σ_d^2, is equal to $4(\sigma_f^2 - \sigma_m^2)$ and the ratio of σ_d^2 to σ_g^2, the quantity of primary interest in this study, is

$$\frac{\sigma_d^2}{\sigma_g^2} = \frac{\sigma_f^2 - \sigma_m^2}{\sigma_m^2} = \frac{\sigma_f^2}{\sigma_m^2} - 1.$$

Procedure in estimation was to estimate σ_m^2 and σ_f^2, and then to substitute estimates of these quantities in the above expressions to obtain estimates of σ_g^2, σ_d^2, and σ_d^2/σ_g^2. Estimates of σ_m^2 and σ_f^2 were computed as follows

$$\sigma_m^2 = (M_1 - M_2)/rln$$
$$\sigma_f^2 = (M_2 - M_b)/rl$$

These are not unbiased estimates. Instead their expectations (value that would be approached in the long run if successive estimates were averaged) are, from expectations of the separate mean squares

$$E(\hat{\sigma}_m^2) = \sigma_m^2 + \sigma_{my}^2/l$$
$$E(\hat{\sigma}_f^2) = \sigma_f^2 + \sigma_{fy}^2/l.$$

However, if it is assumed that the variance from interaction of genotype with environment will on the average be proportional to the variance of the genetic effects involved; in this case that

$$\frac{\sigma_{my}^2}{\sigma_m^2} = \frac{\sigma_{fy}^2}{\sigma_f^2}.$$

Then

$$\frac{\sigma_f^2 + \sigma_{fy}^2/l}{\sigma_m^2 + \sigma_{my}^2/l} = \frac{\sigma_f^2}{\sigma_m^2}$$

and the biased estimates of σ_f^2 and σ_m^2 are as useful as unbiased estimates would be for our purposes. This assumption of proportionality between interaction variance and variance due to direct effects of genotypic variation involved is reasonable on the argument that all kinds of genetic effects (additive or non-additive) must operate through the same sorts of physiological paths and hence may be expected to be equally sensitive to variation in environment. On the other hand it might be argued that σ_{fy}^2/σ_f^2 would be larger than σ_{my}^2/σ_m^2 whenever σ_f^2 was larger than σ_m^2 which would be the case when dominance variance was present. The rationale of this argument would rest on the premise that as variation in genotypic effects increases the general magnitude of interaction effects would increase by a greater than proportional

amount. Intuitively, this seems possible, whereas deviation from proportionality in the opposite direction does not. If this argument is correct, then

$$\frac{\sigma_f^2 + \sigma_{fy}^2/l}{\sigma_m^2 + \sigma_{my}^2/l} > \frac{\sigma_f^2}{\sigma_m^2}.$$

As will be seen later, the possibility of this sort of bias in the ratio between estimates of σ_f^2 and σ_m^2 will have no important bearing on the interpretation of results.

It should be noted that $E[(M_1 - M_2 - M_3 + M_4)/rtn] = \sigma_m^2$
and $E[(M_2 - M_4)/rl] = \sigma_f^2$

and hence these functions of the mean squares would have provided unbiased estimates of σ_m^2 and σ_f^2, respectively. However, though it is not immediately obvious,

TABLE 2

Estimates of additive genetic variance (σ_g^2) obtained with biparental progenies in three open-pollinated varieties of corn

Character	Jarvis			Weekley			Indian Chief
	Sample		Combined	Sample		Combined	
	I	II		I	II		
Date of flowering	3.26	5.36	4.31	3.56	11.45	7.51	4.14
Plant height	35.48	37.28	36.37	26.40	35.24	30.84	33.84
Ear height	21.88	10.92	16.40	28.20	31.00	29.60	26.60
Kernel row number	1.5756	1.4056	1.4908	0.9928	0.8220	0.9072	—
Ears per plant	0.0656	0.0263	0.0460	0.0836	0.0350	0.0593	0.0335
Ear length	0.1448	0.1720	0.1585	0.2580	0.3645	0.3113	—
Ear diameter	0.0066	0.0028	0.0047	0.0058	0.0086	0.0072	—
Yield	0.0044	0.0036	0.0040	0.0032	0.0036	0.0033	0.0024

TABLE 3

Estimates of dominance variance (σ_d^2) obtained with biparental progenies in three open-pollinated varieties of corn

Character	Jarvis			Weekley			Indian Chief
	Sample		Combined	Sample		Combined	
	I	II		I	II		
Date of flowering	1.67	(−1.51)	0.08	0.95	(−6.47)	(−2.76)	(−1.33)
Plant height	6.68	9.64	8.18	4.92	9.68	7.28	(−1.64)
Ear height	6.56	12.92	9.73	8.96	(−0.76)	4.08	(−8.16)
Kernel row number	(−0.2044)	0.3348	0.0648	(−0.0764)	0.0620	(−0.0072)	—
Ears per plant	0.0048	(−0.0030)	0.0008	(−0.0556)	0.0	(−0.0277)	0.0160
Ear length	0.0772	(−0.0408)	0.0181	0.0368	(−0.0611)	(−0.0122)	—
Ear diameter	0.0001	0.0031	0.0015	0.0058	(−0.0010)	0.0024	—
Yield	0.0004	(−0.0012)	(−0.0003)	0.0024	0.0008	0.0017	0.0008

TABLE 4

Ratios of estimates of dominance variance and additive genetic variance, σ_d^2/σ_g^2

Character	Variety		
	Jarvis*	Weekley*	Indian Chief
Date of flowering	.02	**	**
Plant height	.22	.24	**
Ear height	.59	.14	**
Kernel row number	.04	**	—
Ears per plant	.02	**	.48
Ear length	.11	**	—
Ear diameter	.32	.34	—
Yield	**	.52	.33

* Ratios of estimates from sample I and II combined.

** Estimate of σ_d^2 was either zero or negative.

the coefficients of variation of estimates so obtained would have been considerably greater than of the estimates actually made. For this reason the biased estimates were considered preferable, particularly since the effect (if any) on estimate of σ_d^2/σ_g^2 is most likely of a nature that does not affect the primary conclusions from the study.

RESULTS

Estimates of additive genetic variance and dominance variance for the various characters measured in the three varieties are listed in tables 2 and 3. The combined estimates for Jarvis and Weekley were computed from the pooled results of the two samples of progenies in each of these varieties and will be used in subsequent discussion of the estimates for these varieties.

Considering sampling errors to which the estimates are subject, the pattern of results is remarkably similar for different samples of material from the same variety and for the three different varieties. As the experiment was conducted, estimates of σ_d^2 have considerably greater sampling variance than estimates of σ_g^2. The negative estimates of σ_d^2 are well within the range possible through sampling error assuming the true variances to be positive but small. In fact since variances, by definition, are never negative it is most reasonable to conclude that true values of these negative variances are small positive quantities and that the negative estimates resulted from sampling error.

Ratios of estimates of dominance and additive genetic variance are listed in table 4.

DISCUSSION

Comparative magnitudes of additive genetic and dominance variance

Without exception, the estimate of additive genetic variance used in computing the ratios in table 4 was larger than that of dominance variance in the same character. Thus the *a priori* possibility that observation of considerably more dominance than additive genetic variance would provide strong support to the case for importance of overdominance was not realized. The conclusion seems obvious that,

though there may be overdominance at some loci, overdominance loci at which gene frequencies are such that much more dominance than additive genetic variance is produced *are not* the single important source of genetic variation in the varieties studied. This raises the question of possible alternatives, with respect to the genetic situation in these varieties, that are consistent with results of the experiment.

Following symbolism[2] employed by COMSTOCK and ROBINSON, (1948) and (1952), the additive genetic and dominance variances in a randomly breeding diploid population are

$$\sigma_g^2 = \sum_i 2q_i(1 - q_i)[1 + (1 - 2q_i)a_i]^2 u_i^2 \qquad (1)$$

and

$$\sigma_d^2 = \sum_i 4q_i^2(1 - q_i)^2 a_i^2 u_i^2 \qquad (2)$$

if only two alleles are involved at each segregating locus. The subscript, i, identifies the locus so that summation in the above expressions is over all loci. When the frequencies of all segregating genes are one-half,

$$\sigma_g^2 = \tfrac{1}{2} \sum_i u_i^2 \quad \text{and} \quad \sigma_d^2 = \tfrac{1}{4} \sum_i a_i^2 u_i^2$$

and $2\sigma_d^2/\sigma_g^2$ becomes a weighted average of the (a_i^2)'s weighting being relative to the (u_i^2)'s. With this in mind COMSTOCK and ROBINSON (op. cit.) noted that, in the case of populations in which $q = \frac{1}{2}$ could be assumed for all segregating loci, the ratio $\bar{a}^2 = 2\sigma_d^2/\sigma_g^2$ constitutes a meaningful estimate of the average dominance of genes affecting the character in question. It is obvious, on the other hand, that this ratio does not provide a basis for inference concerning level of dominance unless there are satisfactory grounds for assumptions concerning gene frequencies.

The following discussion will have reference to the single character, grain yield, though it will apply in varying part to the other characters on which data were reported. Reasons for this restriction are (a) it is with respect to yield inheritance that importance of overdominance has been postulated by HULL (1945), CROW (1948) and BRIEGER (1950); (b) yield is the one character in corn for which there is any body of experimental evidence that is suggestive of overdominance (see ROBINSON *et al.* (1949), HULL (1952) and GARDNER *et al.* (1953)) and (c) a history of unidirectional selection (for high yield) can probably be assumed. The significance of the third point is that it provides a basis for assumptions concerning gene frequencies. We take the stand that in view of past selection it is reasonable to postulate that in adapted open-pollinated varieties favorable genes are present in higher frequencies than are their less favorable alleles.

Assume as a starting point for discussion that multiple alleles were not present in the varieties studied. Then using equations (1) and (2) the ratio σ_d^2/σ_g^2 for genetic

[2] Let B symbolize the more favorable of two alleles at the i-th locus. Then definition of a_i and u_i is such that the average effects of genotypes classified according to whether they are BB, Bb, or bb at the i-th locus may be represented as $z + 2u_i$, $z + u_i + a_i u_i$, and z, respectively. Clearly, the effect of the heterozygote relative to those of the homozygotes, i.e. the nature of dominance present, is reflected in the value of a_i. q_i is the population frequency of the more favorable allele.

TABLE 5

Values of σ_d^2/σ_g^2 for a single locus, and for various values of a *and* q

a	q				
	.5	.6	.75	.9	.99
.8	.32	.44	.66	.89	.27
1.0	.50	.75	1.50	4.50	49.50
1.5	1.12	2.20	13.50	10.12	.20
2.0	2.00	5.33	∞*	2.00	.08

* When $q = \frac{1+a}{2a}$, $\sigma_g^2 = 0$ and σ_d^2/σ_g^2 becomes infinite. $(1 + a)/2a$ takes a value in the range, 0.5 to 1.0, whenever $a > 1.0$, i.e. when overdominance exists. In such cases it may be called the equilibrium gene frequency since it is the value (or very nearly so) toward which q gravitates in response to intrapopulation selection (unless selection is based on outcross progeny performance).

variance from any single locus can be computed as a function of a and q. A series of values[3] are listed in table 5.

A specific example will help to clarify how the information in table 5 applies. Consider a segregating locus where dominance is complete ($a = 1.0$) and the population frequency of the more favorable allele is 0.9. In terms of genetic variance arising at this single locus, σ_d^2 would be 4.5 times as large as σ_g^2. Thus if the same situation ($a = 1.0$ and $q = 0.9$) prevailed at all loci affecting yield, the value of σ_d^2/σ_g^2 would be 4.5 as compared to the low estimates actually obtained (table 4).

Arguing solely from table 5, three proposals can be advanced in explanation of the experimental results.

(a) That there is no overdominance, *i.e.* $a < 1.0$ at all loci. There is an obvious variety of combinations of $q > .5$ and $a \leq 1.0$ for which the expectation of σ_d^2/σ_g^2 would conform with estimates obtained.

(b) That there is both partial and overdominance, gene frequencies at the overdominant loci being close to "equilibrium" so that these loci are the source of much more dominance than additive genetic variance. The situation visualized is one in which some loci (the ones showing partial dominance) are the source of much more additive genetic variance than dominance variance while others (those showing overdominance) are producing mostly dominance variance, the net result when measured in terms of total σ_g^2 and σ_d^2 from both classes of loci being ratios of the order observed. This is in accord with arguments by CROW (1952).

[3] Only positive values of a are considered. It is reasonable to question whether this is justified. a would be negative in value if the average effect of the *Bb* genotypes were less than the mean for average effects of the *BB* and *bb* genotypes, *i.e.* in the case of what we shall here refer to as negative dominance. The possibility of negative dominance is ignored for the following reasons:

1. The invariable expression of heterosis in crosses of inbred lines in corn is evidence that, at least at the vast majority of loci, dominance is positive.
2. Overdominance has unique implications with regard to the genetic situation in corn only if it is of the positive type. It is selective advantage of the heterozygote that reconciles segregation (intermediate gene frequencies) and ineffectiveness of selection for variety improvement. With negative overdominance the heterozygote is at a selective disadvantage and continued selection should result in a gradual approach to elimination of one of the two alleles from the population.

(c) That there is only overdominance, gene frequencies being close to 1.0 (see lower right corner of table 5).

Of these proposals, the third must be rejected on the grounds that gene frequencies in the necessary range are unlikely in the face of past selection. On the other hand neither general considerations nor the experimental results themselves provide firm grounds for choice between the first two proposals. The situation then is that these results, taken by themselves, are explainable in terms of only partial dominance but are equally compatible with presence of both partial and overdominance.

The foregoing discussion is premised on the assumptions (a) that the estimates of σ_d^2/σ_g^2 were unbiased and (b) that multiple alleles were not present. These issues deserve further comment.

Multiple alleles

FISHER (1918) noted that the definitions of σ_g^2 and σ_d^2 (τ^2 and ϵ^2 in his notation) can be generalized to apply without restriction concerning the number of segregating alleles per locus, and that the way in which σ_g^2 and σ_d^2 contribute to parent offspring and fraternal correlations in random breeding populations is then independent of the length of allelic series. The importance of this generalization is that it gives meaning to σ_g^2 and σ_d^2 that remains consistent whether multiple alleles are or are not present without in any way changing the definitions as they apply in the two allele case.

Speaking now in terms of this broader concept of additive genetic and dominance variance the following can be shown to hold as in the two allele situation.

(a) Assuming equilibrium with respect to linkage relations among loci, no epistasis, and random mating, the variances referred to herein as σ_m^2 and σ_f^2 have the composition

$$\sigma_m^2 = \tfrac{1}{4}\,\sigma_g^2 \text{ and } \sigma_f^2 = \tfrac{1}{4}\,\sigma_g^2 + \tfrac{1}{4}\,\sigma_d^2 .$$

Thus $\dfrac{\hat{\sigma}_f^2}{\hat{\sigma}_m^2} - 1$ remains an appropriate estimate of σ_d^2/σ_g^2.

(b) Effectiveness of intra-variety selection depends on presence of additive genetic variance. Thus observation of considerable additive genetic variance raises the same question relative to possibilities of variety improvement whether or not multiple alleles are considered an important element in the genetic system.

Given two alleles and heterzygote superiority, i.e. overdominance, selection should eventually bring gene frequencies to near equilibrium at intermediate values. This is the base of the argument of those who emphasize overdominance in explanation of the genetic situation in corn varieties. The same sort of equilibrium is possible starting with multiple alleles and, depending on relative values of the homozygous and heterozygous genotypes possible at the locus, the equilibrium state could involve non-trivial frequencies of three or more alleles (see WRIGHT and DOBZHANSKY 1946). However, in any event, loci at such an equilibrium would produce dominance variance but no additive genetic variance. Thus multiple allelism by itself requires no essential change in the interpretation of our estimates of σ_d^2/σ_g^2.

Possible sources of bias in estimates of σ_d^2/σ_g^2

The estimate of σ_d^2/σ_g^2 was computed in terms of the estimates of σ_m^2 and σ_f^2 as $(\sigma_f^2/\sigma_m^2) - 1$, see *Material and Methods*. It was noted that variance due to interaction of genotype with environment is a probable source of bias in $\hat{\sigma}_f^2$ and $\hat{\sigma}_m^2$ but that if the magnitude of bias is proportional to that of the variance estimated there is no net effect on the estimate of σ_d^2/σ_g^2. It was further noted that if biases in σ_f^2 and σ_m^2 from genotype-environment interaction variance were not proportional, there is reason to suspect that, expressed percentage-wise, bias would be greatest in σ_f^2. The effect of this would have been to cause upward bias in the estimates of σ_d^2/σ_g^2.

Another possible source of bias is in the assumed relations:

$$\sigma_m^2 = \tfrac{1}{4}\,\sigma_g^2 \text{ and } \sigma_f^2 = \tfrac{1}{4}\,\sigma_g^2 + \tfrac{1}{4}\,\sigma_d^2$$

COMSTOCK and ROBINSON (1953) list assumptions on which derivation of these relations rests. Multiple allelism has been considered above. Of the remaining, the most suspect are those of no epistasis and no effect of linkage on the relative frequencies of genotypes. Demonstration of possible consequences of epistasis and linkage requires a great deal of space and will be presented elsewhere. It will suffice to state here that when linkage effects are present[4] they operate to make the quantity $\frac{\sigma_f^2}{\sigma_m^2} - 1$ greater than σ_d^2/σ_g^2, and that, with possible rare exceptions, the same seems true for effects of all types of epistasis at least when multiple alleles are absent.[5]

Note that all these possible sources of bias in estimates of σ_d^2/σ_g^2 lead to upward bias. Hence to the extent that any one or more of them is considered of probable imtance, the evidence from the experiment becomes less favorable to the overdominance hypothesis.

Relation to other experimental evidence.

Estimates of the average dominance of genes affecting grain yield reported by ROBINSON *et al.* (1949) and GARDNER *et al.* (1953) were in the overdominance range (values of $\hat{a}$ ranged from 1.31 to 2.14). However, as noted at the beginning of this article, the authors in both instances warned that their estimates were subject to bias from both linkage and epistasis and hence would be considered as indications of an upper limit for average dominance. HORNER (1952) has evaluated the amounts of bias that would result from some specified types of epistasis. His findings are not easily summarized in a few sentences nor, in view of possible linkage effects, is it considered vital to do so here except to state that in the estimates referred to above, linkage is considered of greater immediate concern as a source of bias than is epistasis. COMSTOCK and ROBINSON (1952) demonstrated that upward linkage bias is probable in estimates by the method used by GARDNER *et al.* (1953). Later they also

[4] It was noted earlier that there is reason to believe linkage effects to be minimal in long established varieties.

[5] The consequences of epistasis in multiple allelic systems have not been investigated. While there would appear no special reason to suspect they would differ in pattern between multiple and two allele systems, the issue merits investigation.

showed (1951) that if the number of loci involved in yield inheritance is as great as often assumed, linkage bias in the procedure used by ROBINSON *et al.* (1949) as well as in that used by Gardner and his coworkers would be sufficient so that estimates as large as those reported by these groups of workers are to be anticipated in the presence of no more than partial or complete dominance. Thus the suggestion here that overdominance may not play an important role in corn yield inheritance is not incompatible with other results reported from this station and in a sense receives support therefrom. On the other hand it must be emphasized that the totality of our evidence does not exclude overdominance as a factor of major consequence.

HULL (1952) presents the results of regression analyses of data on yields of inbred lines and the F_1 hybrids obtained from crosses among them, and shows the basis for inference (about level of dominance) from these analyses. Results from 16 of 25 independent sets of data are in accord with the overdominance hypothesis, while those from the other 9 sets are not. HULL points out that sampling errors are large and states that "more comprehensive and precise data" are needed.

ROJAS and SPRAGUE (1952) report estimates of variance components from field comparisons of the possible F_1 hybrids among inbred lines within each of two groups of lines. The lines of a group probably were not derived from the same variety but might be considered a sample from some population having wider boundaries than those of a single variety. Ignoring possible effects of epistasis,[6] what the authors call variance in general combining ability would be equal to half the additive genetic variance of the parent population and their variance of specific combining ability would be the dominance variance of the parent population. Thus their estimates of these two variances[7] provide estimates of σ_d^2/σ_g^2. From their table 3, the estimate from data on group 1 (unweighted analysis) is $1.20/2(1.56) = .38$ and from data on group 2 is .59. SPRAGUE and TATUM (1942) pointed out that if the inbred lines involved in data of this kind have been selected on the basis of previous estimates of general combining ability, the general combining ability variance among them will tend to be depressed. ROJAS and SPRAGUE (1952) indicate that their lines had been subjected to preliminary selection of this kind, which suggests the possibility of an upward bias in the above estimates of σ_d^2/σ_g^2 derived from their results. SPRAGUE and TATUM (1942) give results (see footnote 7) for two sets of lines that were unselected in terms of general combining ability from which derived estimates of σ_d^2/σ_g^2 are .24 and .32, respectively. Admittedly the sampling variances of these and our own estimates of σ_d^2/σ_g^2 are large, and there may be reason to argue that the ratio need not be of the same order for single varieties as for populations of the kind to which estimates derived from the data of ROJAS and SPRAGUE and SPRAGUE and TATUM must be referred. Nevertheless, the general concordance among the several estimates of σ_d^2/σ_g^2 is striking.

[6] Linkage goes unmentioned because its effect here is to increase the sampling error of the specific and general combining ability variances rather than to introduce bias.

[7] The ROJAS and SPRAGUE estimates were so computed as to be free from bias from genotype-environment interaction effects. This was not the case for the SPRAGUE and TATUM estimates.

It is most likely that the effect of epistasis in this kind of data is to contribute proportionately more to the variance in specific combining ability than to the variance in general combining ability and hence to cause upward bias in the derived estimates of σ_d^2/σ_g^2.

The magnitude of additive genetic variance

If overdominance loci with gene frequencies at or near to equilibrium values were the primary source of genetic segregation in these varieties there should be little additive genetic variance. The data indicate, however, that σ_g^2 is of moderate size; large enough in fact so that intra-variety selection should be rather effective. Suppose one selfed a series of plants from one of these varieties and at the same time outcrossed each of these plants to a random sample of other plants of the variety (the sample being a different one for each pollen parent). The result would be a series of half-sib test progenies, one for each pollen parent. Assume further that selection among pollen parents is based on performance of the test progenies in field comparison and that a new population is produced by interbreeding the inbred progenies of selected pollen parents. This is the type of procedure reported by LONNQUIST (1949). It can be shown that, in terms of symbolism of this paper, the expected improvement per generation from such selection is

$$\frac{s\sigma_g^2/2}{\sqrt{\sigma_m^2 + \sigma_{my}^2 + \frac{\sigma_f^2 + \sigma_{fy}^2}{n} + \frac{\sigma_e^2}{r} + \frac{\sigma_w^2}{rk}}}$$

where s is the selection differential expressed as a multiple of the standard deviation of progeny means and n is the number of plants to which each pollen parent was outcrossed (on the basis that each seed parent contributes the same number of offspring to the progeny). The formula as given assumes selection is based on comparison of the progenies in r replications in a single year and location. Substituting variances estimated from our data and setting $r = 6$, $k = 10$, $n = 20$, and $s = 1.3$ (values of n and r are those used by LONNQUIST and $s = 1.3$ is approximately the selection differential to be expected on the average when the best 8 of 36 are selected as in LONNQUIST's experiment), the predicted improvement in yield from selection based on test progeny performance is found to be .054, .045 and .039 pounds per plant for the Jarvis, Weekley and Indian Chief varieties, respectively. Expressed in percent of observed mean yields these become 15%, 12% and 13%.

These represent considerable increments of improvement and presumably improvement could be extended further by selection in recurrent cycles. Actually our estimates of σ_g^2 are subject to upward bias from genotype-environment interaction effects (as noted earlier) and hence the above estimates of effect of selection would be subject to corresponding bias. At the same time it is worth emphasis that in LONNQUIST's selection experiment with the variety Krug the improvement from selection as measured in field comparison of descendants of the inter-bred selected material with the Krug variety was 18% (actually larger than any of the figures given above).

While our estimates of additive genetic variance and LONNQUIST's results both indicate that varieties are susceptible to significant improvement through selection, the long held tenet of corn men to the contrary reflects too much experience by too many capable workers to be ignored. In the opinion of these authors the published evidence falls short of conclusive proof that no further improvement of adapted

varieties is possible.[8] It is recognized, however, that this belief probably rests on much more experience than that actually recorded in the literature. Without insisting that significant varietal improvement is definitely possible, we believe the issue should be re-examined and we have initiated selection programs with the Jarvis, Weekley and Indian Chief varieties to check whether predictions based on data herein reported can be attained.

If selection is indeed ineffective in these varieties a way must then be found to reconcile that fact with the apparent presence of significant amounts of additive genetic variance. Two quite different possibilities will be discussed.

LERNER and DEMPSTER (1951) reported that selection for long shanks in White Leghorn chickens was effective through five or six generations but that further increase in length was not obtained by continuing selection in another five generations. At the same time their data suggested that additive genetic variance in shank length was of the same order of magnitude in late as in early generations of their experiment. A similar picture is reflected in a report by REEVE and ROBERTSON (1953) on results of selection for long wings in *D. melanogaster*. The principal difference is in time relations; wing length increased intermittently through about 46 generations, then remained roughly constant through 30 generations of continued selection, though considerable additive genetic variance in wing length was judged to be present. Though interpretations advanced in the two cases differ in detail, they are similar in essence. Stated briefly and perhaps therefore inadequately, the substance of hypotheses advanced by the two pairs of authors is that if there is negative genetic covariance between a desired trait and fitness (net reproductive rate), selection for the trait may be ineffective even though additive genetic variance exists. The crux of the situation postulated is that the positive force of selection is counterbalanced by lower reproduction on the part of the individuals that are genetically most desirable with respect to the trait under selection. In neither case do the authors claim quantitative fit of observations to hypothesis. Nevertheless their proposal appears entirely reasonable. So long as wing or shank length were subjected to only natural selection it would be expected that genes (or chromosome blocks) that would increase length but decrease reproductive rate would remain rare and be the source of little genetic variance in length. But under direct selection for length the population frequencies of such genes would be increased to the point where they might account for the sort of situations observed. Other genes with effects only on length presumably provided most of the original additive genetic variance, i.e. the basis for early gains from selection. These genes would gradually approach homozygosity as a consequence of selection and the additive genetic variance associated with them would gradually be replaced by variance due to segregation of the originally rare genes (those having positive effects on length but negative effects on reproduction). If so, absence of noticeable change in amount of additive genetic variance would be quite possible.

This same situation may conceivably be present in corn and would account for ineffectiveness of selection even though additive genetic variance in yield is present. However, other things being equal there should be a high correlation between grain

[8] This view was advanced by COMSTOCK and ROBINSON in a paper read at the 1950 Annual Meeting of the American Society of Agronomy.

yield and reproductive rate since each kernel produced represents a potential member of the next generation. (FISHER (1949) suggests as reasonable the equating of chance of survival to yield in grain crops and CROW (1948) and (1952) postulated that an increment in grain yield is associated with a proportional increment in survival value.) Therefore, to make the argument apply in this case it is necessary to distinguish components of reproductive capacity and to point out that certain genes favorable to yield may be unfavorable to such other components of reproductive rate as germination rate and seedling survival. The same argument has, in fact, been presented in these terms by BRIEGER (1950). It is not necessary that the same genes which supply the bulk of the additive genetic variance present also be those primarily responsible for heterosis in crosses of inbred lines (this was implied earlier when we postulated that the additive genetic and dominance variance observed may have been supplied for the most part by two different classes of loci, and is emphasized by CROW, 1952). However, such could be the case if for a locus with dual effects as outlined, a degree of dominance of favorable effects on yield were associated with recessiveness of unfavorable effects on one or more other components of reproductive rate.

Another possibility for reconciling presence of additive genetic variance with ineffectiveness of selection is that gene frequencies at loci providing most of the additive genetic variance are already at equilibrium between the forces of mutation and selection. In this case continued selection would have no net observable effect on mean yield but would operate only to maintain the equilibrium. If such loci had effects only in yield, no single locus would provide very much genetic variance but, if one is willing to assume enough of them, one can account for the amount of additive genetic variance observed. An obvious question is "how many loci must be assumed?" The answer depends upon the level of dominance, magnitudes of gene effects and natural mutation rates. For example, suppose that all additive variance is produced by loci at which gene action is completely additive, that the deleterious effect of the mutant allele at such loci is great enough so that any plant homozygous for the mutant at but one locus is barren even though the plant is homozygous for the favorable allele at all other loci, and that the mutation rate is 1.0 in 100,000. It can be shown that in such a situation the additive genetic variance would be of the order observed in these varieties if the number of loci were about 14 or 15 thousand. Thus an equilibrium of the sort outlined may be either part or all of the explanation for ineffectiveness of selection even though a moderate amount of additive genetic variance is present. Further speculation here concerning the probability that this is the complete explanation is hardly to the point since so very little is known concerning number of loci, natural mutation rates, or the average magnitude of effect of deleterious mutant genes.

SUMMARY

Estimates of the additive genetic and dominance variances in various quantitative characters are reported for three open-pollinated varieties of corn. These were based on data from 512 biparental progenies in each of the varieties. All progenies were grown with two replications in each of two years making a total of 2048 plots

of material from each of the three varieties. Principal features of the findings are (a) the fact that in all characters of the three varieties the estimate of dominance variance was less, and in most instances considerably less, than the estimate of additive genetic variance and (b) the considerable amount of additive genetic variance in grain yield that appears to exist. Implications of the estimates are discussed with primary reference to the character, grain yield.

In view of the smallness (less than 0.6) of estimates of the ratio of dominance to additive genetic variance, it must be concluded that overdominant loci are not the single important source of genetic variability in the varieties studied. The results are explainable in terms of either partial to complete dominance at all loci or a mixture of partial and overdominant loci. Epistasis, linkage, and genotype-environment interaction are potential sources of bias but it appears most likely that all would operate to increase the estimate of dominance variance proportionately more than that of additive genetic variance. Hence if the estimates of σ_d^2/σ_g^2 are biased, the bias is probably upward rather than downward.

While it appears that overdominant loci cannot be admitted as the only important source of segregation for grain yield in corn varieties, this does not imply that overdominance is necessarily insignificant in the genetics of yield. Present evidence must be considered inconclusive in this regard.

Two possibilities are advanced for reconciling presence of additive genetic variance with ineffectiveness of intra-variety selection. The first rests on negative genetic correlation between grain yield and other components of net reproductive capacity. The second envisages the additive genetic variance as arising from loci at which gene action is largely additive and gene frequency is at equilibrium between the forces of mutation and selection. At the same time it is suggested that effectiveness of intra-variety selection should be thoroughly re-examined.

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EVIDENCE AGAINST MICRONUCLEAR MUTATIONS AS THE SOLE BASIS FOR DEATH AT FERTILIZATION IN AGED, AND IN THE PROGENY OF ULTRA-VIOLET IRRADIATED, *PARAMECIUM AURELIA*

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MORTALITY at autogamy in *Paramecium aurelia* increases with the length of the interval since the previous fertilization (PIERSON 1938, GELBER 1938). All stocks of *P. aurelia* studied have characteristic interautogamous intervals, though varying also with conditions (SONNEBORN 1937), and lines maintained past their usual interval without autogamy will be called aged lines. The increased mortality at autogamy in aged lines agrees in general with RAFFEL's hypothesis (RAFFEL 1932) that mortality at endomixis (autogamy) is the result of lethal mutations in the micronuclei: with longer intervals the chances of mutations occurring increase. RAFFEL's hypothesis has been strongly criticized by JOLLOS (1934), and other explanations of the increased mortality can be found in the literature. PIERSON suggested that some at least of the mortality is due to loss of micronuclei from the aged lines. SONNEBORN (1947) suggested a progressive reduction in concentration of cytoplasmic factors that are essential to life but unable to increase as fast as the animals, analogous to the reduction in concentration of kappa particles in rapidly dividing animals. An investigation has been undertaken of the causes of mortality at autogamy in aged lines, with the aim of testing RAFFEL's hypothesis.

OBSERVATIONS ON LINES WITH ABNORMALLY LONG INTERAUTOGAMOUS INTERVALS

For this work stock 90 of variety 1 was used, which has a normal interautogamous interval of around 60 fissions. Aged lines of mating types I and II were selected according to the method of SONNEBORN (1938). Daily isolation lines were maintained and stained samples of each line were examined each day. Lines which went into autogamy were replaced by sister lines which did not. Attempts to conjugate aged animals after 100–150 fissions were unsuccessful, probably because the animals from aged lines went directly into autogamy when their food supply was exhausted, without first becoming sexually reactive.

Aged lines of mating type I were continued, and extensive observations were made on one line after 200–250 fissions (around 100 days) without autogamy. The possibility cannot be completely excluded that over this long period the line had passed through autogamy unobserved. However, the evidence from the stained samples was confirmed by the absence of any temporary drop in fission rate charac-

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TABLE 1

Micronuclei in vegetative animals. Percentage frequency distribution

	Number of micronuclei					Total animals counted
	0	1	2	3	4	
Aged line	8	4	79	8	1	154
Non-aged line	0	4	95	1	0	63

teristic of autogamy, and by the recurrence of autogamy in sublines at intervals too short for another normal interval. Furthermore the fission rate, the appearance at autogamy, and the fate of the exautogamous animals indicated that the line was well aged. Fission rate in the daily isolation cultures, i.e., maximum fission rate, fell from a mean of 3.15 with a standard deviation of .66 fissions per day at 27°C during the first week, to a mean of 1.75 with s.d. 1.00 after 200 fissions. Many individuals of the aged line died spontaneously without passing into autogamy, as observed by SONNEBORN (1938), often surviving several days without fission before death. Micronuclei were examined in stained samples of two cultures, each derived from a separate individual of the aged line. The mean and variation did not differ significantly in the two samples; pooled counts of the micronuclei are shown in table 1, together with a count of a sample from a normal clone of stock 90. Although the mean number of micronuclei in the aged line is only slightly less than normal, there is greater variation in their number. This variation cannot be due to sublines with different stable numbers of micronuclei, since each sample was descended from a single individual after only a small number of fissions. The number of micronuclei must be unstable, with selection no doubt holding the average number approximately constant. Examination of stained samples also provided an estimate of the frequency of spontaneous autogamy in the aged line, since animals for staining were carried for a few days by daily isolation but without selection for non-autogamous individuals. Macronuclear fragments, indicating autogamy or macronuclei in two or more parts, were found in 14.5% and 23.2% of animals in the two cultures examined. These various differences between aged and normal lines show that the micronuclear mutation hypothesis, which may account for mortality at autogamy, cannot account for the effects of aging in the vegetative animal. RAFFEL considered variations of this type, which arise in the absence of fertilization, to be due to mutations in the macronucleus.

MORTALITY AT NUCLEAR REORGANIZATION

Mortality at autogamy in stock 90 after the normal interautogamous interval is 4%–10%. In the aged line mortality at autogamy was very high. For estimation of mortality, small cultures of animals from the aged line were allowed to exhaust their food supply. Stained samples were then examined for autogamy. Living animals in autogamy could not be selected, since their appearance during autogamy was unusually variable. Single animals were isolated from autogamous cultures into a limited quantity of culture fluid. After five days the isolations were examined, and only clones which had exhausted their food supply were considered viable. Viability

measured in this way is somewhat arbitrary, and exautogamous clones which were scored as non-viable might occasionally have been able to pass through further fissions if left a longer time or if reisolated into a fresh supply of food. However, the number of fissions which isolated exautogamous animals from less aged lines passed through after five days were sharply bimodal: generally the food supply was exhausted, or else division stopped after a few fissions. Ninety six animals were isolated from each of three cultures of the aged line which contained only autogamous animals in stained samples. The groups isolated showed 100%, 100%, and 98% mortality. The survivors in the last group may not have passed through autogamy, since their descendants went into autogamy after exhausting their food supply in the viability test. Or, since after macronuclear regeneration another reorganization recurs spontaneously after a short period, an autogamy could have taken place at which the macronucleus regenerated from fragments. When more extensive samples were tested for viability by serial isolation as described below, a small fraction of individuals from aged lines (less than 1%) were found to survive autogamy.

CYTOLOGICAL OBSERVATIONS ON NUCLEAR REORGANIZATION

For further study of autogamy a method was needed of inducing autogamy simultaneously in all animals in a culture. With the usual method of allowing a culture to exhaust its food supply, it was found that some animals of the aged line went into autogamy before the food supply was fully exhausted, and these might have passed through a post-autogamous fission before the rest of the culture went into autogamy. Consequently the method was adopted of isolating all the fission products of a single individual, and then reisolating all their fission products at daily intervals. Several hundred animals were collected after a few days and transferred, after one or two washings, into suitably diluted Ringer's solution. Using this method on control stock 90 (approximately 60 fissions after the previous autogamy), it was found that a culture which initially contained no autogamous animals, contained 39% after 8 hours' starvation, 71% after 20 hours, and 72% after 32 hours. Starvation for 20 hours was therefore adopted with the aged line.

Animals from the aged line went into autogamy rapidly and variably. After 8 hours' starvation in dilute Ringer's 30% to 50% were in autogamy. At this time 70% of the autogamous animals in the control group were in the skein stage, but more than 90% of the aged were past this stage. The proportion in autogamy after 20 hours' starvation was found from examination of mass cultures, and also of individuals which were isolated and underwent fission. The range in different cultures was from 36% to 96%, with a mean of 78%. Examination of samples showed that the aged group after 20 hours' starvation was scattered through various stages of autogamy.

The breakdown of the macronucleus could be followed in the stained preparations. No difference between animals from the aged line and controls was noticed at the skein stage. Fragments of the macronucleus were counted in animals starved for 20 hours, as shown in table 2. This frequency distribution is bimodal. Animals with fewer than 30 fragments may have undergone more than one fission during the period of starvation, but this group is more likely to be animals already in autogamy before

starvation, since the proportion of animals in this group is similar to the frequency of exautogamous animals in unstarved samples. The group of animals containing more than 40 fragments includes all those with macronuclear anlagen, and clearly comprises autogamous animals which have not undergone post-autogamous fission. The mean number of fragments in this group is 51.8 ± 8.1, to be compared with 37.9 ± 5.8 fragments in autogamous control animals. The fragments in animals from the aged line did not appear smaller than normal. The excessive number of macronuclear fragments indicates disturbance of autogamy in animals from the aged line at the time before fertilization has taken place. The disturbance could not therefore have been brought about by a new genotype produced at the autogamy.

The larger number of macronuclear fragments in the aged autogamous animals might be taken as evidence for a vegetative, not an autogamous disturbance: for example the possibility that the macronucleus is larger in vegetative animals has not been excluded. This distinction is formal, since disturbances at or soon after autogamy in aged lines can hardly be shown to be unconnected with earlier vegetative changes. Since disturbances in nuclear processes arise under the influence of the

TABLE 2

Autogamous animals from aged lines

(a) Appearance of autogamous animals

Number of macronuclear fragments							Total animals counted
0–10	10–20	20–30	30–40	40–50	50–60	60–70	
6	4	1	0	10	16	4	41

Number of macronuclear anlagen								
1	2	3	4	5	6	7	8	
1	9	10	8	1	3	0	1	33

Number of micronuclei									
0	1	2	3	4	5	6	7	8	
1	1	13	3	3	0	0	3	0	24

(b) Relation between number of macronuclear anlagen and number of micronuclei

Number of micronuclei	Number of anlagen						
	0	1	2	3	4	5	6
0		1					
1						1	
2			7	4	2		
3			1	2			
4					2		1
5							
6							
7				1	1		1

original genotype, death at autogamy may likewise be independent of the new genotype.

Micronuclei and macronuclear anlagen could be counted in late stages of autogamy, and considerable variation was found, as shown in table 2 (a). Thirty-three control autogamous animals with anlagen were examined, and all contained two micronuclei and two anlagen. The formation of an abnormally large or small number of nuclei presumably occurs before nuclear differentiation since the numbers of anlagen and micronuclei are related to one another, as shown in table 2 (b). The positive correlation is significant at the 1% level, although the counts of micronuclei in these animals is uncertain.

MORTALITY AND MACRONUCLEAR REGENERATION FOLLOWING NUCLEAR REORGANIZATION

Exautogamous clones from the aged lines were studied by isolating single animals from mass cultures starved for 20 hours. Single animals were stained and examined when possible after the first and third fissions, or after the second if they failed to undergo a third fission. Any surviving clones were then propagated by daily reisolation. Data on the exautogamous clones are grouped in table 3. A proportion of the stained first fission products did not appear to be exautogamous, and in repeated experiments this proportion was in close agreement with the proportion of non-autogamous animals in stained samples of the original mass cultures. Consequently the whole group of isolated animals that died undivided can be considered as autogamous, and figure 1 is calculated accordingly. It could not be ascertained whether animals that died before the second fission, together with a few of those that died after, formed the new macronuclei from anlagen or from fragments of the old macronucleus by macronuclear regeneration. Among the remaining animals undergoing nuclear reorganization, 16.2% went through macronuclear regeneration. Abnormal behaviour of the nuclei before the formation of anlagen could not have caused this high frequency of macronuclear regeneration, since few animals examined before the first post-autogamous fission contained less than two anlagen. Furthermore, normal animals were often found among the other third fission products of a

TABLE 3

Macronuclear regeneration and mortality after autogamy in aged lines

	Number of fissions till death							
	1	2	3	4	5	6	6	Totals
No nuclear reorganization								251
Undivided								490
Autogamous:								
Macronucleus developed from anlagen (normal development)	0	66	51	33	9	9	28	196
Macronucleus regenerated from fragments macronuclear regeneration)	0	11	19	5			3	38
Source of macronucleus doubtful	105	5						110
Total number of isolations observed								1085

single individual showing macronuclear regeneration. The four third fission products examined were twice found to contain 0, 1, 1, and 2 macronuclei, with the individual containing no macronucleus showing macronuclear regeneration. In such a case mis-segregation of the macronucleus may have occurred at the third fission.

The mortality among the exautogamous lines showing macronuclear regeneration was as high as the mortality among lines showing normal development of the macronucleus. In the macronuclear regeneration lines the mortality cannot be the result of micronuclear lethals, since the new macronucleus is not derived from the micronuclei. The new macronucleus in these cases may possibly differ from the old as a result of segregation of macronuclear mutations, but there is no evidence that segregation of this sort can occur. If death in these cases is not due to the new genotype, and if the alterations in aged lines which cause death after macronuclear regeneration occur with equal frequency among animals which show normal macronuclear development, these alterations provide a sufficient cause of death among the lines with normal macronuclear development. However the possibility that the newly formed genotype in lines with normal macronuclear development may also be lethal is not excluded by this argument.

Post-autogamous mortality is shown graphically in figure 1 with undivided animals included in the autogamous group. Among animals undergoing reorganization, 3% passed through more than six fissions. Most of these animals gave rise to weak lines which died out after a few fissions, but two were successfully carried to a second autogamy. With the estimate of the number of animals that did not divide after autogamy omitted, the curve of mortality in figure 1 is exponential and gives no indication

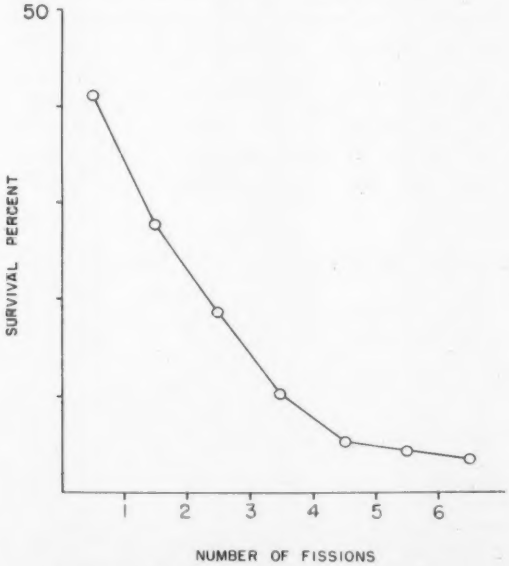

FIGURE 1. Post-autogamous survival of aged lines

of cytoplasmic lag of post-autogamous death. If there were cytoplasmic lag, the exautogamous animals might be expected to multiply for some fissions with little or no death, and then show a rise to a peak of mortality and then a falling off. The absence of evidence for cytoplasmic lag can be interpreted as evidence against death being due to the genotype of the newly formed macronucleus. On the other hand, as recessive lethal mutations accumulate, mortality would to an increasing extent be due to those which act more rapidly after autogamy. Consequently the high mortality observed in this experiment weakens the argument against the micronuclear mutation hypothesis from the absence of cytoplasmic lag. The data on the number of fissions preceding death in clones undergoing macronuclear regeneration indicate that in these lines the processes leading to death may be different.

INHERITANCE OF CHANGES INDUCED BY ULTRA-VIOLET IRRADIATION

Complete genetical analysis of the changes occurring in aging lines could not be made because the aged animals could not be crossed with normal animals. Attention was therefore directed towards an agent which might mimic the effects of aging without preventing conjugation. Ultra-violet radiation was the agent selected. Ultra-violet irradiation of vegetative paramecia causes mortality at the next autogamy (KIMBALL and GAITHER 1951). In view of the evidence from X-radiation (KIMBALL 1949), the major genetic effect of ultra-violet was expected to be induction of recessive lethal and detrimental mutations in the micronuclei. However, little is known about the details of conjugation following irradiation, and this conjugation provides a method of detecting genetic effects of radiation other than gene mutations. Evidence can be cited from work with other microorganisms that ultra-violet radiation causes more cytoplasmic changes than X-radiation, in proportion to its efficiency in producing gene mutations (HOLLAENDER, SANSOME, ZIMMER, and DEMEREC 1945). An experiment was carried out in which a previously irradiated and genetically marked stock was crossed with an untreated stock, and the exconjugants analyzed genetically

STOCKS

Two stocks of variety 4 were used in this experiment. The first stock, here called stock A (not the stock designated stock A in SONNEBORN's collection), was derived from stock 51, but with its antigen genes for antigens A, F, and H replaced by alleles a^{29}, f^{29}, and h^{29} from stock 29, and its killer gene K also replaced by gene k from stock 29. Stock A is no longer maintained, but the same genic combination is available in another line. Stock d2a13 was also derived from stock 51, but with its antigen gene for antigen D replaced by allele d^{32} from stock 32. These two stocks were otherwise essentially isogenic with stock 51, for after each foreign gene was introduced there were at least 7 successive backcrosses to stock 51 before the separate isogenic stocks were combined to give multiple markers. These two stocks were prepared by DR. T. M. SONNEBORN and his co-workers and were made available to the author for the present experiments. The genetic formulae for these two stocks, showing the loci at which they differ, are:

Stock A: $a^{29}, a^{29}, d^{51}, d^{51}, f^{29}, f^{29}, h^{29}, h^{29}, kk$
Stock d2a13 $a^{51}, a^{51}, d^{32}, d^{32}, f^{51}, f^{51}, h^{51}, h^{51}, KK$

EXPERIMENTAL PROCEDURE

Animals of stock A, mating type VIII, were sent into mass autogamy in tube culture. Cultures showing 100% autogamy were given enough food to permit three fissions, and animals from them were irradiated when the food supply was nearly exhausted. Approximately 125 animals in 0.1 ml culture fluid were deposited for irradiation in a glass depression slide. The dosage was for three minutes at 40 cm. from a GE germicidal lamp, 15 watt. The dosage under these conditions was 3291 ergs/mm^2 (measured by DR. H. BUTZEL). Single animals were isolated after irradiation and left in darkness for 24 hours, manipulations being carried out in yellow light to prevent photo-reactivation. After the post-irradiation cessation of division, single descendants of the irradiated animals were isolated every two days, to carry on a line of descent. When more than 90% of the animals left over from each line were found to be in autogamy, usually after the second isolation, 96 autogamous animals were isolated and their viability tested. Ability to exhaust a limited quantity of culture fluid was used as the test of viability, following the procedure described above for aged lines. Following the nomenclature of KIMBALL (1949) this exautogamous generation will be called the A1. Eight separate lines of descent from irradiated animals were tested in this way, and showed a mean mortality of 65.4% Unfortunately this figure is of little value, since an undetermined fraction of the A1 died in a characteristic fashion, probably as a result of some contaminant and not of the radiation.

Meanwhile the descendants of the single animals isolated after irradiation were allowed to pass through approximately 10 fissions and to become sexually reactive. Their antigenic type was then determined, and each culture was mixed with unirradiated animals from stock d2a13 of mating type VII and of a different antigenic type. Conjugating pairs were isolated after 4 hours, due precautions being taken to select only pairs which were tightly joined. When the pairs separated, the two members were put into separate containers, and the exconjugant derived from the untreated stock detected by addition of the appropriate antiserum. The antigen of the untreated animal was tested for, in order to ensure that the viability of the exconjugant of irradiated ancestry would not be affected by the antiserum. Groups of sixteen pairs were taken from each of eighteen lines of descent from single irradiated animals. The generation of the exconjugant clones will be called the BC. Products of the third fission after conjugation of each clone in fifteen of the groups were examined for macronuclear regeneration. The BC clones were continued by isolation every two days. When more than 90% of the animals left over were found to be in autogamy, a set of 48 isolations of autogamous animals was made. Autogamous cultures showing the same contamination as the A1 could be recognized and were discarded, and though only 75 sets of isolations were finally analysed, non-specific death did not appear to affect the results. This exautogamous generation will be called the A2.

The A2 clones were left for five days to determine their viability. Sixteen viable clones were then taken from each set (except for one set, derived from macronuclear regeneration at conjugation, which gave no viable clones). These clones were tested for the killer character. Some sets of clones descended from the stock A conjugant proved to be all sensitive, and some from the stock d2a13 conjugant all killer. These

sets were further tested for segregation of the antigens A^{29} and A^{51}. This further test was necessary, since the gene *K* might have been segregating in some sets of sensitive clones descended from the stock A conjugant, but the segregation would not have been detected if kappa had not been transferred at conjugation. Undetected segregation of *K* was expected in A2 clones descended from the stock A conjugants only when the corresponding A2 set of clones descended from the d2a13 conjugant showed segregation of the killer character. Consequently only 6 clones in each set were tested for the antigen, and the expectation was fully verified. All sets of A2 clones descended from the d2a13 conjugant which failed to show segregation were A^{51}, and all from the stock A conjugant were A^{29}. From the data on the killer tests of the A2, it was found that kappa had passed into the sensitive conjugant in 19 of the 27 conjugating pairs tested.

EXPERIMENTAL RESULTS

The results of this experiment are shown in table 4. Each column in this table is divided in two, according to whether the animals were derived from the irradiated stock or from the untreated stock, as determined by the antigenic marker at conjugation. In the first column the pairs of exconjugant BC clones are divided into four classes, "+" indicating survival of the clones, and "−" indicating death. The amount of macronuclear regeneration as determined at the third fission after conjugation is shown in the second column. The proportion of non-viable clones showing macronuclear regeneration is recorded, since some clones died out after the third fission, as well as some before. The majority of clones in which macronuclear regenerations occurred proved to be non-viable. The third column shows whether segregation was detected in the combined killer and antigen tests on the A2 generation, "+" indicating segregation, and "−" indicating no segregation. The segregation data were in accord with Mendelian expectation and are not recorded here extensively. Mortality of the A2 clones is recorded in the final column.

There are certain features of these results which need explanation. These are:—

(1) Exconjugant clones of irradiated ancestry had a higher probability of death (45/288) than exconjugant clones of normal ancestry (10/288). This result cannot be attributed to chance, $\chi^2_{(1)} = 246$, $p < 10^{-6}$.

(2) When both members of a pair of BC clones proved viable and gave rise to sets of A2 clones, either both showed segregation or neither did, i.e., no cases were detected where the A2 set derived from the irradiated animal showed segregation and the paired set from the untreated animal did not, and vice versa. The observed result cannot be due to some effect preventing segregation which operates independently in the members of a conjugating pair for the chance against the 4 non-segregations among the 31 untreated pair members being paired with 4 non-segregations from the irradiated members is less than 10^{-3}.

(3) When the member of a pair of BC clones of irradiated ancestry died or showed macronuclear regeneration, the other member of the pair failed to show segregation after autogamy (13/13). This difference cannot be attributed to chance. This frequency can be compared with the pairs where both clones proved viable, when the frequency of failure of segregation after autogamy was 4/31. $\chi^2_{(1)} = 12.5$, $10^{-3} > P > 10^{-4}$.

TABLE 4

Analysis of conjugation between clones descended from irradiated and untreated animals, and of the following autogamy

Viability of BC clones (exconjugant viability)		Macronuclear regeneration in exconjugants		Segregation at autogamy		Mortality of A2 (exautogamous mortality)	
Irradiated	Untreated	Irradiated	Untreated	Irradiated: from exconjugant with normal macronuclear development	Untreated	Irradiated	Untreated
+	+81.9% (*288*)	2.0% (*198*)	0.0% (*198*)	+	+87% (*31*)	6.9% (*1296*)	5.8% (*1296*)
				−	−13% (*31*)	4.2% (*192*)	0.5% (*192*)
				Irradiated: from exconjugant with macronuclear regeneration	Untreated		
					−100% (*1*)	100% (*48*)	0.0% (*48*)
					Untreated		
−	+14.6% (*288*)	25.0% (*32*)	3.1% (*32*)		−100% (*12*)		5.6% (*432*)
+	−2.4% (*288*)	0.0% (*7*)	0.0% (*7*)				
−	−1.1% (*288*)	0.0% (*3*)	0.0% (*3*)				

Figures in brackets following percentages indicate totals from which the percentages were calculated.

These observations cannot be explained solely by the induction of mutations in the micronuclei of irradiated animals. Such mutations are expected to be distributed equally between the members of an exconjugant pair, and for the most part are not expected to be manifest before the A2.

Feature (1) above, the higher mortality among the BC clones of irradiated ancestry, suggests a radiation effect on the cytoplasm or macronucleus which causes death at nuclear reorganization. An effect could be exerted at conjugation following irradiation either by cytoplasmic lag (i.e., a transient effect), or by cytoplasmic or macronuclear inheritance (i.e., a persistent effect.). This kind of effect is analogous to the changes in aging lines described earlier and might have been expected in this experiment. But if such an effect were the sole cause of death at conjugation, and occurred equally frequently whether cross-fertilization occurred or not, untreated animals should have shown segregation in the A2 generation after conjugation with irradiated animals which died as well as well as after conjugation with irradiated animals which lived. This hypothesis therefore provides no explanation of feature (3) above.

CYTOGAMY

The observations can be accounted for by a high frequency of cytogamy at conjugation. Pairs of exconjugant clones failing to show A2 segregation could be produced by cytogamy when both exconjugant clones proved viable. High mortality among the BC clones of irradiated ancestry could be the result of induced lethal mutations becoming homozygous at cytogamy, and would account for the normal member of the pair's failure to show A2 segregation. Similarly failure of A2 segregation on the normal side of the exconjugant pair coupled with macronuclear regeneration on the irradiated side could be the result of cytogamy, on the hypothesis that homozygous lethal anlagen fail to function properly and the failure is in some cases early enough for the old fragments to take over. However the evidence is not at all conclusive for this mechanism of macronuclear regeneration.

An unusually high frequency of cytogamy in stock 51, which has a normal frequency of less than 1%, is necessary if it is assumed that no other mechanism operates during conjugation of the irradiated stock. And on the same assumption, a high mortality among the members of the conjugating pairs of irradiated descent must follow cytogamy (a figure of 49% mortality can be calculated). However radiation may well induce other defects besides cytogamy at the conjugation.

MORTALITY AT AUTOGAMY FOLLOWING CONJUGATION AFTER IRRADIATION

The mean mortalities of the descendants of both members of non-cytogamous pairs at autogamy is given in the top line of the last column in table 4. In accordance with genetical expectation, this mortality is approximately equal among descendants of either member of the pairs, and the overall mean mortality is 6.3%. The symmetrical distribution of this mortality between the autogamous descendants of the two members of the pairs indicates that recessive micronuclear mutations are the chief irradiation effect persisting into the A2. A control value for this mortality is provided by the mortality at autogamy of animals descended from cytogamous pairs. Recessive

lethal mutations are eliminated at cytogamy, so that mortality at the following autogamy is independent of the irradiation before conjugation. Mortalities among autogamous animals descended from cytogamous pairs are enclosed in a dotted line in table 4, and these are in the range 0.0%–5.6%. After subtracting these control values the mortality of the A2 of irradiated ancestry is in the range 0.7%–6.3%, so that the A2 mortality resulting from the irradiation is barely significant.

This mortality is surprisingly low, in comparison with the mortality postulated at cytogamy. If death is the result only of fully lethal micronuclear mutations becoming homozygous, and with the low observed A2 mortality, the mortality at cytogamy among animals of irradiated descent should be no more than approximately double the A2 mortality. The comparatively low A2 mortality can be accounted for by a sufficiently small number of lethal mutations in comparison with detrimental mutations which exert a lethal effect only in combinations (KIMBALL 1949), and data are not available on the comparative effect of ultra-violet radiation in producing lethal and detrimental mutations. The comparatively low A2 mortality is also suggestive of an increased likelihood of death among animals of irradiated descent after their first nuclear reorganization.

Similar effects are not brought about by X-radiation (KIMBALL 1949). In crosses between descendants of irradiated and untreated animals low mortalities were found among exconjugant clones. However one experiment showed a higher mortality, which KIMBALL suggests may have been the result of cytogamy. The values of the A1/A2 ratio were calculated in this work, and values were found approximately equal to 2.

If the cytogamy hypothesis put forward here is correct, ultra-violet irradiated animals resemble in certain ways the CM stock investigated by METZ and FOLEY (1949). Both the CM and the ultra-violet irradiated animals manifest a block to normal conjugation, and the origin of the CM stock indicated that it differed from related clones only in its complement of cytoplasmic factors. Although conjugation pairs are not formed within this stock, it can form pairs with normal animals. However, the block to normal conjugation occurs at an earlier stage in CM x normal pairs than in cytogamous pairs after ultra-violet irradiation, since the CM x normal pairs are joined only by their cilia and can be separated mechanically. In these pairs the normal member undergoes a process genetically identical with cytogamy, while the CM member fails to undergo macronuclear fragmentation, meiosis, or fertilization.

DISCUSSION

Evidence has been presented that changes in the genetic complement of the micronuclei cannot easily account for the increased mortality at autogamy in aged lines of paramecia. On the contrary, the changes at autogamy can be more easily accounted for by a changed cytoplasm or macronucleus, or both. A distinction between effects in the macronucleus and cytoplasm cannot be drawn from these experiments. Evidence has also been presented that the simple micronuclear mutation hypothesis cannot account for all the events at the fertilization following ultra-violet irradiation, and it is suggested that in this case also damage is caused to the cytoplasm or macronucleus. With both the aged and the irradiated animals the evidence for death not due

to micronuclear mutations is indirect. The micronuclear mutation hypothesis can certainly be extended to cover all the observations here reported, although some unlikely assumptions might be required. And, while the evidence against this hypothesis is not strong, the evidence for any other particular mechanism is weak.

The micronuclei have been shown not to function during vegetative growth (SONNEBORN 1946). If the evidence against the micronuclear mutation hypothesis is accepted, the rest of the cell would appear to accumulate damage during vegetative growth. The symmetrical A2 mortality found in the radiation experiment indicates that the postulated somatic damage disappears after nuclear reorganization, at the same time as a new macronucleus is formed. This provides a reason for the evolution of autogamy additional to its purely genetical function of rendering the animal homozygous for all genes. Autogamy could be described as a means for providing an opportunity at regular intervals for the soma to be formed afresh from the germ line.

MEDAWAR's hypothesis of aging (MEDAWAR 1951) can be extended to explain the origin of periodic nuclear reorganization. Consider a hypothetical population composed of animals whose probabilities of entering nuclear reorganization is independent of the number of fissions elapsed since the last nuclear reorganization. The lengths of lines of vegetative descent will be distributed exponentially in the population, and comparatively few animals will have long intervals elapsed since their last reorganization. Consequently genes which produce deleterious effects only after long intervals of vegetative fission will be eliminated by selection less rapidly than genes which act more rapidly. An example of this kind of delayed expression is provided by loss of kappa in stocks of Variety 2 caused by high fission rate (PREER 1946). This type of delayed effect must not be confused with cytoplasmic lag, which is of a different nature. As the genes which act more rapidly are eliminated the population will change, so that as the number of fissions after nuclear reorganization increased, the chances of eventual extinction also increase. Selection pressure therefore favors shorter intervals between reorganization. Periodic reorganization is therefore selected for. Furthermore, once a periodicity has been established, genes exerting deleterious effects after the normal inter-reorganization interval are shielded from natural selection. An accumulation of such genes will increase the selection pressure for periodicity of reorganization, and will tend to shorten the period.

Evidence can be found in the literature that conjugation fulfils the same role as autogamy in the production of a new macronucleus at regular intervals, among Holotrichs which do not undergo autogamy. In *Paramecium aurelia* there is a long period after nuclear reorganization when autogamy can only be induced with difficulty, but conjugation can generally take place after fewer fissions. Similarly *P. caudatum* undergoes regular autogamy (ERDMANN and WOODRUFF 1916), but no long immature period after conjugation has been reported (GILMAN 1941). Attempts to detect autogamy have failed in *P. bursaria* (WOODRUFF 1931), and *Tetrahymena gelei* (NANNEY 1953), and these species both show a long immature period after conjugation (*P. bursaria:* JENNINGS 1939; *T. gelei:* NANNEY and CAUGHEY 1953). The situation is complicated by inter-stock differences; nevertheless a long immature period seems to be associated with the nuclear reorganization expected to be responsible for somatic renewal.

SUMMARY

1. Autogamy after unusually long inter-autogamous periods, and conjugation after ultra-violet irradiation are described in *Paramecium aurelia*.

2. After unusually long inter-autogamous intervals in Variety 1 mortality at autogamy is increased. The macronucleus breaks down into a larger number of fragments. The numbers of micronuclei and macronuclear anlagen are variable. Variations from normal autogamy commence before any new genotype formed at autogamy is expected to act.

3. A high frequency of macronuclear regeneration is found in these autogamies. After macronuclear regeneration the mortality is as high as among animals in which the macronucleus develops from a micronucleus.

4. No evidence is found for cytoplasmic lag of post-autogamous mortality after these autogamies.

5. Conjugation of the progeny of ultra-violet irradiated animals with normal animals is described, making use of genetically marked stocks of variety 4. Exconjugant clones of irradiated ancestry have a higher probability of death than exconjugant clones of normal ancestry. When both members of a pair of exconjugant clones are viable and give rise to sets of clones by autogamy, no cases are found in which one set shows genetic segregation and the other does not. When the number of irradiated descent from a pair of exconjugant clones dies or undergoes macronuclear regeneration, the other member of the pair fails to show segregation after autogamy.

6. These observations are discussed, and it is concluded that mutations in the micronuclei are unlikely to be the sole cause of death after fertilization under these experimental conditions. This conclusion is discussed in relation to the evolution of periodic nuclear reorganization in Holotrichs.

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THE PRODUCTION OF MUTATIONS IN DROSOPHILA MELANOGASTER WITH CHEMICAL SUBSTANCES ADMINISTERED IN SPERM BATHS AND VAGINAL DOUCHES[1]

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OF ALL the techniques so far employed in the field of mutagenesis, vaginal douches given just before insemination provide the shortest pathway for the chemical to take in order to reach the chromosomes (HERSKOWITZ 1947, 1949a). In addition, the cells treated in this manner are uniform in that all the sperm are known to be mature at the time of exposure. Although this technique was successful in inducing sex-linked recessive lethal mutations not only in sperm but in eggs of *Drosophilia melanogaster* after treatments with a nitrogen mustard solution, it did not raise the mutation rate for either gamete when sublethal solutions of formaldehyde or certain other chemical substances were introduced (HERSKOWITZ 1949a). The negative results may have been obtained for a number of reasons, including the possibility that the gametes were treated by too small an amount of chemical for an insufficient period of time. That the technique for administering this chemical was sometimes inadequate is not indicated by the failure of formaldehyde to produce mutations in eggs after vaginal douches since this substance has failed to mutate female germ cells in all studies thus far reported regardless of the method of treatment (HERSKOWITZ 1950; AUERBACH 1951, 1952b; AUERBACH and MOSER 1953a; VALENCIA 1951; RENDEL and ROBERTSON, unpublished, but quoted by AUERBACH and MOSER 1953a), but is suggested from recent experiments with formaldehyde which have been successful in inducing mutations in sperm that were probably mature at the time of treatment (AUERBACH 1952a). For this reason, two additional methods were devised for treating sperm that have been deposited in females. These procedures, the sperm bath and postcopulatory vaginal douche, were employed with formaldehyde as well as urethane, trypsin, ammonia, copper sulfate, and the nitrogen mustard, methyl bis (beta-chloroethyl) amine hydrochloride.

The methods used for treatment are described and the results of tests for sex-linked recessive lethal mutations following various treatments are presented. The data and some general considerations concerning the genetic effects that are dependent on the nature of a mutagen and the manner in which it is administered are discussed in detail.

MATERIAL AND METHODS

In the absence of a feasible technique for chemical treatment of sperm *in vitro* followed by artificial insemination, the precopulatory vaginal douche technique had

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been employed earlier (HERSKOWITZ 1947, 1949a). The present work uses two new modifications of this procedure. In precopulatory and postcopulatory vaginal douches the vagina of an adult female is flooded with a chemical substance either just before copulation or some hours after mating. In the former case the sperm mass is exposed directly to whatever portion of the chemical substance is retained by the female at the time of sperm ejaculation. In the latter case the sperm are already stored in the seminal receptacles of the female at the time of treatment, and, unless the chemical can enter the vaginal openings of these receptacles, the substance must first pass through the walls of the receptacles in order to reach the sperm.

The sperm bath method treats the sperm mass directly by bathing the sperm with a chemical substance soon after they are deposited in the vagina. Preliminary experiments showed that sperm are introduced into the vagina about 8–9 minutes after copulation is initiated. Until about 12 minutes from the start of copulation no sperm had entered either the ventral receptacle or the spermathecae, and almost an hour elapsed before all the sperm had migrated from the vagina to the storage organs. Accordingly, females were etherized 9 minutes, or within a few minutes later, from the start of copulation (at which time the males usually were still mounted on the females), and placed dorsal side down on the stage of a dissecting microscope. A pair of coarse forceps (which was held by a ring-stand and whose movement was regulated by an adjustable screw) was used for compressing the abdomen laterally, causing the eversion of the vagina. The milky ball of sperm within can now easily be seen, and a fine glass pipette filled with the chemical solution to be used was inserted into the vagina, and the liquid forced on the sperm. That at least some of the sperm were actually in direct contact with the chemical solution was evident, for some of the sperm often adhered to the pipette at the time it was withdrawn from the vagina. Although at the time of treatment almost all, if not all, of the sperm are in the vagina, it is probable that different sperm in the same vagina are exposed to varying concentrations of the chemical substance. It should also be noted that even with this technique the pathway taken by the chemical substance is indirect, since it includes going through whatever fluids surround the sperm, the cellular membranes enclosing the chromosomes in the sperm head, and whatever small amount of cytoplasm may be contained therein. All three methods are very simple to perform and treating more than a hundred females a day is not at all difficult.

X chromosome recessive lethal mutations following treatments with various chemical substances were detected by the standard "Basc" procedure (MULLER *et al* 1954), starting with males of the Oregon-R, wild-type stock and females of the Basc (formerly called the "Muller 5") stock. Accordingly, lethals detected in the F_2 generation originated in mature sperm of the Oregon-R stock and in eggs of the Basc stock. A lethal mutation, detected by the absence of Oregon-R or Basc males, or both, in the F_2 or a subsequent generation, was considered certain once 20 or more males of the non-lethal type appeared. All but four of the lethals reported were established in this manner. Of these, one P_2 female had lethals on both X chromosomes, and three (table 9) did not provide quite the 20 sons of the non-lethal type required.

Trypsin, ammonia and copper sulfate (table 1) were used in solutions made with

distilled water, the N mustard (table 7) was dissolved in 0.75% NaCl or in saline (prepared according to BUCK and MELLAND 1942), and urethane (table 1) was dissolved either in saline or tap water. For the treatments listed in table 3, solutions of 10, 5, 4, and 2% formaldehyde were made by dilutions with saline, 3% solutions with tap water, 2.5% solutions with either tap water or 0.75% NaCl. Control solution "(1)" was made of fly feces mixed with a coarsely filtered water extract of crushed adults, while treatment "3.3 (1)" was the same except that the liquid also contained 3.3% formaldehyde. Control solution "(2)" was made of a coarsely filtered water extract of crushed adults and food medium previously churned by larvae, while treatment "2.5 (2)" was a 2.5% solution of formaldehyde of either this liquid or of tap water containing fresh yeast. Control solution "(3)" was made of a coarsely filtered water extract of food medium which had been seeded with live yeast, while treatment "1.5 (3)" was the same containing 1.5% formaldehyde. Control solution "(4)" was the same as control solution "(2)", and treatment "1 (4)" was the same but contained also 1% formaldehyde. Except for copper sulfate, all "solutions" were used within 8 hours of their preparation. In the case of the "control" and the corresponding formaldehyde mixtures at least one hour elapsed between the times of preparation and use.

Treated females were transferred periodically to vials containing fresh food medium. All work was done at 25 ± 1°C.

RESULTS

The fate of 374 females treated with solutions of urethane, trypsin, ammonia, and copper sulfate administered as sperm baths and postcopulatory vaginal douches is

TABLE 1

Fate of females treated with chemical substances given as sperm baths and postcopulatory vaginal douches

(Values for the latter treatment are in parentheses)

Chemical	Conc., %	Date	No. ♀♀			
			Treated	Dead in 48 hrs	Ovipositing	Producing adult offspring
Urethane	50	5 53	14	6	5	5
	25	6 53	20 (15)	1 (5)	15 (7)	13 (3)
	25	7 53	17	7	9	7
	10	5 53	11	0	7	7
Trypsin	conc. sol.	7 53	60	28	20	17
Ammonia	2.7	7 53	69	44	16	15
Copper sulfate	4	7 53	24	23	0	0
	1	7 53	45	32	6	4
	0.5	7 53	38	18	9	5
	0.25	7 53	61	25	21	14

TABLE 2

Recessive sex-linked lethal mutations obtained, following treatment with sperm baths, from the females in table 1

(Results following postcopulatory vaginal douches are in parentheses.)

Chemical	Conc., %	No. mutant sperm / No. Ore-R sperm tested	No. mutant eggs* / No. Basc eggs tested
Urethane	10, 25, 50	2/1320	4/1050
	25	(0/149)	(0/149)
Trypsin	conc. soln.	0/358	0/358
Ammonia	2.7	1/280	0/280
Copper sulfate	1, 0.5, 0.25	0/523	4/523
Sperm bath totals		3/2481	8/2211

* Includes only 2 lethals or less from a treated female.

summarized in table 1. Table 2 gives the results of tests for sex-linked recessive lethals for the survivors of these treatments. Only 3 lethals were found in 2481 Ore-R sperm tested, and 8 lethals in 2211 Basc eggs tested following the sperm bath treatments, while no lethals appeared in 149 sperm or an equal number of egg chromosomes tested following postcopulatory vaginal douches.

The fate of 113 sperm-bathed and 51 vaginally douched females treated with control solutions, as well as 543 sperm-bathed and 331 postcopulatory-douched females treated with solutions of formaldehyde is presented in table 3, while table 4 sum-

TABLE 3

Fate of females treated with chemical substances given as postcopulatory vaginal douches and sperm baths

Chemical	Conc., %	Date	Sperm baths				Postcopulatory vaginal douches			
			No. treated	No. dead in 48 hrs.	No. ovipositing	No. producing adult offspring	No. treated	No. dead in 48 hrs.	No. ovipositing	No. producing adult offspring
Formaldehyde	10	5/53	15	7	2	2				
	5	5/53	14	8	3	3				
	4	6/53	33	9	18	12				
	3.3 (1)	11/53	37	30	4	2				
	3	9/53	255	58	140	118	242	41	148	137
	2.5	11/53	37	20	12	11				
	2.5 (2)	11/53	53	34	12	10	35	17	13	12
	2	5/53	13	4	8	8				
	1.5 (3)	11/53	26	14	8	7	16	1	12	11
	1 (4)	11/53	60	20	24	22	38	11	16	16
Control for formaldehyde	(1)	11/53	24	11	9	7				
	(2)	11/53	26	12	8	6				
	(3)	11/53	16	5	8	7	15	2	11	10
	(4)	11/53	47	13	24	19	36	2	27	26

TABLE 4

Recessive sex-linked lethal mutations obtained, following treatment with postcopulatory vaginal douches and sperm baths, from the females in table 3

Chemical treatment	Conc., %	Sperm baths		Postcopulatory vaginal douches	
		No. mutant sperm / No. Ore-R sperm tested	No. mutant eggs* / No. Basc eggs tested	No. mutant sperm / No. Ore-R sperm tested	No. mutant eggs* / No. Basc eggs tested
Formaldehyde	10, 5, 4	5/206			
	3.3(1)	0/11	0/11		
	3	9/2381	4/2338	9/3808	13/3618
	2.5	2/228	0/228		
	2.5(2)	5/189	0/189	1/338	2/338
	2	5/229			
	1.5(3)	0/99	0/99	0/182	0/182
	1 (4)	5/941	2/941	2/696	1/658
Total formaldehyde		31/4284	6/3806	12/5024	16/4796
Controls for formaldehyde	(1)	1/153	0/153		
	(2)	0/78	0/78		
	(3)	1/342	1/342	1/419	2/341
	(4)	2/702	0/702	3/1221	1/1221
Total controls		4/1275	1/1275	4/1640	3/1562

* Includes only cases of 2 lethals or less from a treated female.

marizes the results of 23,662 chromosomes tested for lethals among the survivors of these treatments.

Tables 5 and 6 are summaries of the results in tables 2 and 4 for X chromosome lethals in Basc eggs and Oregon-R sperm, respectively.

Table 7 shows the fate of 432 sperm-bathed and 181 postcopulatory-douched

TABLE 5

*X chromosome recessive lethals in Basc eggs**

	No. mutations / No. eggs tested	%	No. lethals from different treated ♀♀	
			1	2
Postcopulatory douches				
A. Formaldehyde	16/4796	0.33	11	2
B. Other (Tables 2, 4)	3/1711	0.18		
Sperm baths				
C. Formaldehyde	6/3806	0.16	16	2
D. Other (Tables 2, 4)	9/3486	0.26		
Total B + C + D	18/9003	0.20		

* Including only cases of 2 lethals or less from a treated female.

TABLE 6

X chromosome recessive lethals in Oregon-R sperm

	No. mutations / No. sperm tested	%	No. lethals from different treated ♀♀		
			1	2	3
Sperm baths					
A. Formaldehyde	31/4284	0.72	19	3	2
B. Other (Tables 2, 4)	7/3756	0.19			
Postcopulatory douches					
C. Formaldehyde	12/5024	0.24	21	1	0
D. Other (Tables 2, 4)	4/1789	0.22			
Total B + C + D	23/10,569	0.22			

TABLE 7

Fate of females treated with N mustard, methyl bis (beta-chloroethyl) amine hydrochloride, administered in sperm baths and postcopulatory vaginal douches

Conc., %	Date	Sperm baths				Postcopulatory vaginal douches			
		No. treated	No. dead in 48 hrs.	No. ovipositing	No. producing adult offspring	No. treated	No. dead in 48 hrs.	No. ovipositing	No. producing adult offspring
4	11/53	135	90	18	0				
2	7/53	32	26	2	0				
2	11/53	16	7	5	1	39	12	20	2
1	11/53	118	64	46	9	27	8	17	4
0.5	11/53	41	17	18	7	27	9	15	12
0.25	11/53	52	27	20	12	38	9	28	21
0.1	11/53	38	17	12	9	50	6	36	29

TABLE 8

Sex-linked lethal mutations obtained following treatments (11/53) with the N mustard, methyl bis (beta-chloroethyl) amine hydrochloride

Conc., %	Precopulatory vaginal douches*				Postcopulatory vaginal douches				Sperm baths			
	Sperm		Egg		Sperm		Egg		Sperm		Egg	
	No. lethals / No. tested	%	No. lethals / No. tested	%	No. lethals / No. tested	%	No. lethals / No. tested	%	No. lethals / No. tested	%	No. lethals / No. tested	%
10	7/127	5.5	4/113	3.5								
4	23/1416	1.6†	23/2537‡	0.9								
2, 1, 0.5, 0.25					49/713	6.9	1/713	.14	9/210	4.3	3/210	1.4
0.2	4/867	0.5										
0.1					26/752	3.5	4/752	.53	7/169	4.1	0/169	0.0

* From Herskowitz (1947, 1949); all ♀♀ copulating within 2 hr. after injection.

† 0.45% (7/1535) from copulations delayed more than 2 hr.

‡ Contains 18/649 (2.8%) for eggs laid the first 2–3 days after treatment.

TABLE 9

The distributions of lethal-bearing sperm (no. lethals/no. sperm tested) among sperm from the same copulations used in fertilization on different days after postcopulatory vaginal douches with N mustard

Conc., %	Days after treatment					
	1–2		2–5		5–8 or more	
0.10%	4/54	7.4%	14/279	5.0%	8/178	4.5%
0.25%	2/31	6.5%	15/161	9.3%	11*/80	13.8%
0.50%	1/25	4.0%	10/114	8.8%	7/61	11.5%

* Includes 3 probable lethals.

females and table 8 gives the results of 3688 chromosomes tested for lethal mutations following treatments with different concentrations of N mustard. The numbers of lethal-bearing and non-lethal-bearing sperm (only from those copulations tested which gave mutations) that were used to fertilize eggs in different periods following post-copulatory vaginal douches are given in table 9.

DISCUSSION

Specific considerations

Mutability tests of urethane, trypsin, ammonia and copper sulfate after sperm bath treatments using sub-lethal concentrations were negative (table 2). It should be noted that, in other experiments, when sub-lethal concentrations of urethane were added on top of the food only a very weak mutagenic effect was detected among the sperm tested from Oregon-R males developing on the treated food medium (HERSKOWITZ and SCHALET, unpublished). The reasons for the negative or weakly mutagenic activity of urethane in our experiments and those of DIPAOLO (1952) in contrast with the strongly mutagenic activity of this substance when applied in the same or similar manner by RAPOPORT (1947) and VOGT (1948) are not clear. Although this substance as well as the others mentioned may have given negative results for reasons of lack of penetration, low concentration or insufficient duration of treatment, or a low mutability of the Oregon-R stock, one other possible explanation should be mentioned. Some substances may be mutagenic entirely, or almost entirely, because they act on the genes only when these take part in metabolism. At such times the genes are involved in interchanges of chemical substances with other parts of the protoplasm, and may have a less protective chemical envelope or a different physical configuration than they have at other times. Since, in mature sperm, the genes are known to be inactive (MULLER and SETTLES 1927) and may have, in addition, a different envelope or configuration than they have at other times, it would not be surprising to find the chromosomes in the sperm immutable to a substance which is highly mutagenic for the same chromosomes when present in other cells of the individual.

It was with just such considerations in mind that earlier studies were made with 2,4-dinitrophenol, in a preliminary attempt to elucidate the types of metabolic activity in which the genes take part (HERSKOWITZ 1949a,b). For if different sub-

stances fail to produce mutations in sperm chromosomes but are mutagenic when given to cells in which genes are active, then, from a comparison of the mutagenic capacities, a start would have been made not only toward identifying the metabolic processes in which the genes partake but also their sequence relative to the genes. At present, to help prove the existence of mutagens which act only on metabolizing genes, the douche and sperm bath techniques must be employed and negative results obtained. In those cases where mutations do occur following chemical treatment of mature sperm, it may be possible to show that the mutations were induced, by a more or less direct action, in inactive genes, leading us towards a better understanding of the chemical composition of the hereditary material.

The negative results obtained after treatments with metabolic wastes ("controls" (1) (2) and (4) in table 4) do not support the idea that naturally occurring or biologically produced mutagens are concentrated and eliminated from larvae or adults.

Before considering further the incidence of recessive lethal mutations in the Basc egg chromosome it should be explained that prior to all postcopulatory vaginal douches any mature egg blocking the opening into the ovary was removed. This procedure made sure that the passageway to the ovary was not occluded, so that eggs could be treated as well as the sperm. In sperm baths, eggs are almost certainly untreated, since the sperm ball completely fills the vagina and it is unlikely that enough chemical is retained in the vagina which could mutate eggs, the first of which pass through it only after a number of hours following copulation have elapsed. It can be seen in the tables that the number of mutation tests of Basc chromosomes is often less than the number tested for the Oregon-R chromosome. This is because the number of Basc chromosomes tested from a given Basc female was subtracted from the total tested in an experiment whenever this female herself proved to be heterozygous for an X chromosome recessive lethal. In addition, no data were included for the Basc chromosome for any female which gave rise to more than two Basc lethals. Although, in so doing, one is discriminating against the detection of a mutagen which is more effective on immature than on mature oocytes, this conservative procedure was followed to avoid falsely claiming a positive mutagenic effect for a chemical when many of the mutations obtained could have resulted from mothers being mosaics for lethal mutations. When a chemical is strongly mutagenic such a correction would not change the decision, but when a chemical is of doubtful mutagenicity there may be an obvious deviation from a Poisson distribution for the number of lethals detected from different mothers, and since mosaicism for a lethal mutation is a rare event (yet each one included would, in our case, add at least 3 lethals to the total), very large samples would be required in order to be sure the spontaneous frequency of mosaics in the controls adequately represented that in the experimental crosses.

On the reasonable assumption that treatments with "control" mixtures, sperm baths containing formaldehyde, and all those substances in table 2 did not *reduce* the spontaneous mutation rate for the Basc chromosome, one can compare the rates obtained from these separately and together with that obtained following postcopulatory vaginal douches containing formaldehyde (table 5). It is clear that there is no evidence formaldehyde increases the mutation rate in egg X chromosomes. This

may not, however, be taken as proof that formaldehyde is incapable of inducing recessive lethals in eggs. For, although it seems unlikely, if the other treatments had *raised* the mutation rate by some amount, then any increase in mutation rate due to formaldehyde up to this amount would have been undetected. There might also be some validity to the idea that some of the lower concentrations of formaldehyde, being of lesser mutagenic potency, have caused the total mutation rate to drop to a value that cannot be shown to be different from that for the "control". By appropriate modifications of the available techniques it may be that formaldehyde will prove mutagenic in eggs, and until additional tests have been made it seems unjust to state formaldehyde is incapable of mutagenic action in this cell.

Turning to the induction of X chromosome lethals in the Oregon-R sperm, it can be seen that there was no detectable increase in mutation rate following postcopulatory vaginal douches with formaldehyde over either the spontaneous rate of 0.2% previously well-established for this chromosome, or the rates obtained from the other sperm baths and douches not containing formaldehyde listed in tables 2 and 4. However, the very first sperm baths with 10, 5, 4 and 2% formaldehyde gave a mutation rate of 2.3% (table 4; HERSKOWITZ 1953). In subsequent experiments, sperm baths with formaldehyde did not yield so high a mutation rate (21/3849, or 0.55%; table 4), although there was a statistically significant difference ($P < .005$) between this and the combined rate (23/10,569, or 0.22%) obtained for Oregon-R chromosomes treated in other ways in concurrent experiments (table 6). The lower rate in the last group of formaldehyde sperm bath experiments performed can only be partly explained by the fact that they include some treatments with formaldehyde concentrations which were lower than those used in the earlier experiments. Nevertheless, there is no doubt that fully mature sperm mutate after sperm bath treatments with formaldehyde.

The data in table 9 are like those of AUERBACH (1952b) for mustard gas in that no significant increase in the frequency of lethals was detected upon ageing of sperm, treated in the present case by N mustard. However, the irregularities of the data are such that a moderate effect of this kind is by no means excluded. If a large number of lethals are delayed mutations, and the evidence is clear that at least some of these are delayed, then one might have expected the number of whole (as distinguished from mosaic) lethals to have been considerably increased by ageing. (AUERBACH and MOSER 1953a, found a statistically non-significant increase in lethals upon ageing sperm following formaldehyde treatments.) That ageing had no clear-cut effect on mustard-gas induced lethals, but had a clear-cut effect in increasing the proportion of all visible mutants (whole plus mosaic) which were whole mutants (AUERBACH 1952b) is an apparent contradiction that has not yet been resolved. However, the second criterion (proportion of mosaics among visibles) may be a more sensitive one, in consideration of the fluctuations to which the first criterion (the total frequency of lethals) is subject.

For ease in comparison, table 8 includes the results with N mustard obtained in the present work as well as some reported previously (HERSKOWITZ 1947, 1949a). Although in the present work all sperm treated were Oregon-R and all eggs Basc, the sperm and eggs treated in the earlier work were of both types. Since the spontaneous

and chemically-induced sex-linked recessive lethal mutation rates for these stocks showed no detectable difference in any of this work, it is not necessary to eliminate from consideration tests made on Basc sperm and Oregon-R eggs in the earlier work before comparisons are made between the two groups of experiments. Several things should be mentioned about the earlier results with precopulatory vaginal douches. When sperm entered the vagina within a 2-hour period following a douche with 4% N mustard their mutation rate was 1.6%, but if they entered after this period, and probably did so within 24 hours after the females were treated, their mutation rate was only 0.45%. The egg X chromosome also mutated after 10% and 4% N mustard precopulatory vaginal douches. In the latter treatment, the increased mutation rate is apparent only in the eggs oviposited the first 2–3 days after douches, when it was 2.8%.

When 135 sperm baths were given using 4% N mustard no offspring were obtained, and only when the dosage was reduced to 1% were there more than a few offspring from sperm bath or postcopulatory douche treatments. Although the actual cause is unknown, the more concentrated N mustard sperm baths may have failed to produce offspring because the sperm were killed and/or forcibly ejected from the vagina by the female (this region being considerably irritated by the mustard as evidenced sometimes by the female rubbing this area with her legs, or dragging it along a surface). However, sperm ejection is the less likely explanation for the failure to obtain numerous offspring following postcopulatory douches where, prior to treatment, the sperm are already stored in the seminal receptacles. When 0.1% N mustard was given either in postcopulatory vaginal douches or sperm baths, a sperm mutation rate was obtained which was as high as that produced by 40–100 times this dose when administered as a precopulatory douche. If only a small amount of a solution is retained in the vagina even during the first two hours following a douche, it would be expected that stronger concentrations can be applied in precopulatory than in postcopulatory douches or sperm baths. This probably explains the situation regarding the eggs, in which no evidence of an increased mutation rate was obtained following postcopulatory douches (or sperm baths) but in which mutations were produced after the higher concentrations of N mustard were administered in douches prior to copulation. Since one would expect that higher concentrations are required to mutate eggs than sperm, it is reasonable to believe that when sperm are already present in the receptacles of the female a sufficiently concentrated dose to be mutagenic to the eggs would result in the death or loss of the sperm. Thus, of the methods mentioned, the best way to obtain egg lethals with N mustard is to give females precopulatory vaginal douches with strong concentrations and wait a sufficient period (say a day) before permitting copulation. On the other hand, sperm lethals are obtained with N mustard with greater ease with postcopulatory than with precopulatory vaginal douches.

Since there is no gross difference in the rates of mutation in sperm treated with the same concentrations of N mustard after sperm baths and postcopulatory vaginal douches, no preference can be given to one procedure over the other on the basis of the data so far available. Now while all of the methods mentioned for mutating mature sperm have proved successful for N mustard, only the sperm bath method has

succeeded in raising the sperm mutation rate when formaldehyde was used. On the reasonable assumption that, on the average, equivalent volumes of these solutions are administered when they are applied by a given method, one can mention some of the factors possibly responsible for the different results obtained with N mustard and formaldehyde. Because N mustard produced mutations in sperm after postcopulatory vaginal douches while formaldehyde did not, although the same concentrations of both were able to produce mutations in sperm when applied in sperm baths, and since the concentrations of formaldehyde which can be applied as postcopulatory douche or sperm bath treatments without killing the females are as high or higher than are possible for N mustard, it is reasonable to believe that N mustard has a general penetration power that is better than formaldehyde's. Although this is consistent with the lower mutation rate induced in sperm baths by formaldehyde as compared with N mustard for treatments of equal concentration, as well as the failure and success, respectively, of these chemicals to mutate female germ cells, there are other factors, besides poorer penetrating ability, which may be important in explaining the lower mutagenic efficiency of formaldehyde (or other chemical substances).

Suppose formaldehyde and N mustard passed through cell membranes with equal ability but that the former substance was so reactive that it never (or rarely) remained uncombined long enough either to reach the chromosomes or to leave a cell it had once entered, while the latter substance could do either or both of these more frequently. One would then expect formaldehyde to give negative and N mustard positive results for sperm lethals in postcopulatory treatments since in this method most of the mutagen reaching the sperm probably must first go through the walls of the receptacles. Accordingly, the observed difference in mutability between the two might have resulted largely from differences in the frequency of occurrence, outside of the genes, of chemical groups with which they combine. However, since the amount of non-genic material subject to interaction with mutagens in mature sperm is the minimum amount known for cells, it may be possible using sperm baths to obtain evidence whether the chemical grouping of the gene mutated by one agent is more, equal or less frequent than another grouping mutated by a different agent.

Strictly speaking, the potency of chemical mutagens can best be compared when they attack the same chemical grouping in the genes. For, if only one-eighth of the genes have a grouping changeable by one mutagen while one-half have a grouping changeable by another mutagen, then even when the two mutagens penetrate as far as the chromosomes in equivalent amounts the latter mutagen will be detected as being four times as powerful as the former.

General considerations

Since comparisons are often made between physical and chemical mutagens and between chemical mutagens themselves, it seems desirable to consider in more detail some of the factors involved in the distribution of chemical substances to the cells studied as well as within them, for such factors may influence the types and frequencies of the mutations that are subsequently detected.

The delivery of an X-ray dose for mutagenic purposes is usually accomplished

(in Drosophila, for example) in a period of an hour or less. In this case the X-ray agent causing the mutations is introduced very close to the places which are mutated, and the mutagenic "hits" are distributed among the cells virtually at random.

This is not the situation when chemicals are administered as mutagens. Not only may the time of exposure be longer than an hour (making a difference in the mutation rate obtained if during the period of treatment the cells to be tested have changed their susceptibility to the chemical), but there is a non-random distribution of the chemical among the cells later tested for mutations. This is so because no matter what technique is used for administering a chemical, different cells (or nuclei) must be closer or further away from the agent which brings the mutagen to them. The result is that some cells are exposed to more and others to less chemical than expected on a random-distribution basis.

The affinity of a chemical substance for certain components of the cell influences not only the amount of chemical distributed to different cells, but also the distribution of its molecules within a cell. The possible pathways a molecule of mutagen may take to reach a chromosome are limited, either because the mutagen is distributed unequally around the cell or because nongenic substances are present in the cell which can destroy the mutagen by interacting with it. Once a molecule has taken a pathway which reaches a chromosome it is more likely for a second molecule to follow this same pathway rather than some other one by which it could reach a chromosome.

It would seem to follow, that when two breaks (or other mutations) occur in the chromosomes of a cell these are likely to be closer together in chemical mutagenesis than they are when produced in a cell by X-rays. All the available data are consistent with this in showing that the ratio of gross intra- to interchromosomal rearrangements found for X-rays is shifted in favor of the former type of mutation when chemical mutagens are employed (dibenzanthracene, DEMEREC 1948; mustard gas, SLIZYNSKA and SLIZYNSKI 1947, AUERBACH 1950; 1:2, 3:4-diepoxybutane, BIRD and FAHMY 1953; 2:4:6-tri(ethyleneimino)-1:3:5-triazine, FAHMY and BIRD 1953; formaldehyde, AUERBACH and MOSER 1953b).

Now because a greater proportion of cells are unhit in the case of a chemical mutagen than of X-rays, then, given the same total number of mutagenic hits for the two, one would find the chemical mutated a lower percentage of cells than did the X-rays. In this respect a chemical is "less efficient" than X-rays.

It is fully realized that the commonly employed practice of comparing the efficiency of two mutagens in producing gross chromosomal rearrangements, using for comparison dosages which give the same frequency of sex-linked recessive lethal mutations for both, may be misleading. Such lethals are not a homogeneous group, for some arise without connection with breakage while others are dependent upon breakage for their occurrence. If two mutagens produce exactly the same genetic effects except that one produces more point mutation lethals this one would be classified incorrectly as mutagenically "less efficient" in comparisons having equivalent lethal rates as the reference point.

Destruction of cells by somatic effects, e.g. a toxic action, is known to take place for some chemicals; with mutagenic ones, it would take place more often in the cells also having mutations and more rarely in those which have none. Thus, somatic

killing would reduce the percent of viable cells with mutations whenever a toxic chemical is used. Killing having a genetic basis, e.g. dominant lethality, would work in the same way to reduce the apparent mutation rate. Any mutagen which has a toxic non-genic effect on the cells treated will be expected to reduce the frequency of multi-hit-type mutations more than one-hit-types among the survivors. Yet on the other hand, as already discussed, the unevenness of the chemical's distribution itself favors multi-hit in relation to one-hit mutations. Where the balance will lie in any given case could hardly be predicted. Until the non-genetic toxicities of chemicals in the cells studied are known it will be difficult to make meaningful comparisons of mutagenic potencies of different mutagenic agents based on the ratios of one-hit to multi-hit mutations, or of two types of mutations when one is a mixture of one and two or more hit events.

The considerations discussed suffice to call attention to the multitude of causes not only for negative results in chemical mutagenesis, but for differences in the kinds and rates of mutation induced by different mutagens, as well as differences in the mutations obtained with the same agent administered in different ways. Since even when substances are administered in sperm baths the treatment is indirect, in the several respects already mentioned, requiring caution in deciding the basis for the results obtained, use of more indirect methods of treatment (or of mixtures of cells) provide results that are more difficult to interpret.

Although the douche and sperm bath methods are not the best of all the techniques available, for all substances under all circumstances, they possess several advantages. A single F_2 culture can be used to test for lethals in both the egg and sperm X chromosomes which entered the F_1 female. Both of these chromosomes may be treated or one may be treated and one not, depending on which technique or combination of techniques is used. In all cases only mature sperm are present, permitting the study of a variety of modifications of physiological, physical and chemical conditions under which a mutagen is operating. No other method in Drosophila permits the investigator, at the start, to place chemical substances so close to the gene.

SUMMARY

The sperm bath and vaginal douche techniques for the treatment of mature sperm and eggs of *D. melanogaster* with chemical substances are described, and their advantages and limitations discussed.

Negative results were obtained from a total of 2481 sperm chromosomes tested for sex-linked recessive lethal mutations following sperm bath treatments with urethane, trypsin, ammonia and copper sulfate.

Formaldehyde was definitely mutagenic to sperm treated in sperm baths, but not when applied in postcopulatory vaginal douches. No evidence was obtained that eggs were mutated by this substance following postcopulatory vaginal douches.

Sperm baths and postcopulatory vaginal douches with N mustard were highly mutagenic to sperm, but did not mutate eggs probably because the concentrations were not as high as those used in previously reported precopulatory vaginal douche experiments which gave positive results. When the frequencies of sperm lethals were scored on the basis of the lengths of time N mustard-treated sperm were held in the

females, no significant evidence was obtained for delayed mutation, although the results do not exclude some effect of this kind.

Some factors involved in the distribution of chemical substances to as well as within the cells studied are discussed in relation to their effect on the types and frequencies of detected mutations.

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THE EFFECTS OF ENVIRONMENTAL TEMPERATURE ON COAT COLOR IN DIVERSE GENOTYPES OF THE GUINEA PIG[1, 2*]

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Although the physiological genetics of guinea pig coat color has been studied from the point of view of gene interaction in the amount of pigment produced (HEIDENTHAL 1940, RUSSELL 1939, WRIGHT 1941, 1942, 1949, WRIGHT and BRADDOCK 1949) and from a biochemical basis (FOSTER 1952, GINSBURG 1944, RUSSELL 1939), no attack on the problem by means of an investigation of the interaction of the genotype with the environment seems to have been made previously except for some studies of albino guinea pigs by ILJIN (1927) and SCHULTZ (1922) and preliminary experiments by WRIGHT (1927) on a number of colored genotypes. In various papers WRIGHT (1949 and previous) has reported the observation that the intensity of the guinea pig hair color changes over a period of time but whether this was due to an age effect, the environmental temperature or to a combination of these or possibly still other factors was not known.

The investigations of the effects of cold on the pigmentation of the Himalayan rabbit by DANNEEL (1941), DANNEEL and PAUL (1940), DANNEEL and SCHAUMANN (1938), ENGELSMEIER (1937), ILJIN (1926, 1927, 1931), KAUFMANN (1925), and SCHULTZ (1922, 1928) and the pronounced darkening of the ears, nose, and feet of certain albino guinea pigs in addition to the appearance of dark hairs in these animals after losing part of their coat pointed to a possible parallelism in the effects of cold on pigment production in these two species.

The purpose of the present study was to discriminate between changes in pigmentation that do and do not depend on a reduction of the skin temperature.

Previous measurements of the intensity and quality of guinea pig hair pigment were made by means of visual grades (WRIGHT 1949) or by colorimetrically measuring the pigment extracted from weighed samples of hair by solution in dilute alkali (HEIDENTHAL 1940, RUSSELL 1939, WRIGHT and BRADDOCK 1949). Although measurements obtained by these methods agree quite well (WRIGHT 1949), the visual grades are not objective for sufficiently small differences for the present purpose.

The Photovolt photoelectric reflection meter, used in obtaining the data to be reported here, makes objectivity possible in the measurement of small qualitative and quantitative differences (in the sense of order) in the pigment present in the

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hairs of the living animal since a photoelectric cell measures the amount of light reflected at three different regions of the spectrum. It does not, however, measure the actual amount of pigment.

MATERIALS AND METHODS

Data on about one thousand guinea pigs of approximately sixty genotypes from Professor SEWALL WRIGHT's guinea pig colony were obtained over a period of two years. However, quantitative comparisons will be made for only twenty-eight genotypes as the number of animals available in the others was insufficient for this purpose.

Four categories of experimental and control animals of each genotype were set up, the distribution of animals into the four categories being randomized as much as possible: (1) animals whose rumps were plucked and exposed to 16°C, (2) unplucked control animals exposed to 16°C, (3) animals with plucked rumps exposed to 32°C, and (4) unplucked control animals kept at 32°C. Environmental temperatures were maintained at 16°C $\pm$ 1°C and 32°C $\pm$ 1°C in constant temperature rooms with constant circulation of air. The animals were kept in darkness during the experimental periods (thirty days) except for a maximum of about one hour of light daily for feeding, watering, and cleaning. Their diet, consisting of Rockland Guinea Pig Diet and water plus cabbage and carrots each once a week, was the same as that of the entire colony.

Plucking was done manually, as much as possible of the hair being removed from the rumps of the experimental animals. It was not possible to remove new hairs which were just beginning to appear above the skin surface and this may be the cause of the appearance of red and yellow hairs with eumelanic subterminal bands to be discussed later.

For obtaining objective measurements of the guinea pig coat colors a Photovolt photoelectric reflection meter, Model 610, was utilized. This device was used by BLAIR (1947) to measure the pelage color of mouse skins but as far as the author knows had not, until now, been used on living animals. The reflection meter consists of a galvanometer and a search unit containing a light bulb and a photoelectric cell. A series of enamel standards varying from pure white to dark gray are used to standardize the readings. The light bulb furnishes the light which is thrown on the surface whose color is to be measured. By means of three filters (amber, green, and blue) which are placed between the measured surface and the photoelectric cell, readings of the amount of light reflected at different wave lengths are obtained. The light reflected by the surface onto the photoelectric cell causes the latter to produce an electric current which moves the galvanometer needle in proportion to the amount of reflected light. In order to get comparable readings the galvanometer needle was adjusted to a reading of 100 (which is equivalent to the amount of light reflected by the enamel standard being used) before each measurement. In addition to a pure white standard (for yellow and white animals) and a light gray standard (for sepia, brown, and black animals) a dark gray standard and a black box were used to check the instrument before each use. The readings of the latter two objects were compared to the white or light gray standards when these had been adjusted to a galvanometer reading of 100. This procedure was very valuable in checking the reflection meter for accuracy and prevented inaccurate readings due to mechanical failures.

In order to have a flat surface for making readings a glass slide was placed over the flat part of the rump of the animal and the opening of the search unit placed on the slide. The slides decreased the readings uniformly.

To make all readings, both those against the white standard and those against the gray standard, comparable and to correct for the loss in reflected light due to the glass slides PROFESSOR WRIGHT prepared tables (unpublished) by means of regression analysis for the conversion of all readings to values comparable against the white standard.

Three readings, one each with the amber, green, and blue filters, were made on each animal before being plucked and/or placed in the constant temperature room and again thirty days later. After thirty days the hair in the plucked area had been replaced and in most cases was visually indistinguishable in length or thickness from the unplucked hair. In those genotypes where no perceptible color change occurred it was impossible to distinguish the experimental from the control animals after thirty days except by their ear punches. Readings were made without knowledge of the genotype and the experimental category into which each animal had been placed.

Genotypes were determined from a combination of the pedigrees and WRIGHT'S (1949) color grades. No animals in which there was doubt as to the genotyge (especially with regard to the C-series alleles) were used in obtaining the data reported here.

Subcutaneous temperature measurements were made weekly for a period of thirty days with a calibrated thermocouple (EGGERT 1946) of copper and constantan. A small channel was made under the skin with a steel nail which had been filed flat, sharp, and pointed. The thermocouple was then inserted into this channel and the reading made on a potentiometer. Twenty-four plucked animals, more or less equally divided as to sex and eu- or phaeomelanic coat color were used. Twelve were kept at 16°C and twelve at 32°C. In addition, control measurements were made on twelve unplucked animals after a one-week exposure to the experimental temperatures.

Hair growth measurements to determine any differences in the rate of hair growth due to plucking or clipping were made by plucking the hair from one-half of the rump of twenty-four animals, more or less equally divided as to sex and eumelanic or phaeomelanic coat color, and clipping the hair on the other half of their rumps as closely as possible with hair clippers. Twelve of these animals were placed into the constant temperature room held at 16°C and the rest were kept at 32°C. Measurements were made every other day for thirty days with a steel ruler divided into millimeters.

The average age of the animals used in the subcutaneous temperature and hair growth measurements was within the age range of the animals used in the coat color experiments.

RESULTS AND DISCUSSION

Rate of hair growth

The rate of hair growth after plucking or clipping and at environmental temperatures of 16°C and 32°C is about 0.5 mm per day which is the same as in man (CHASE 1954) and the rabbit (ILJIN 1927). The new hair of plucked animals appears above

the skin surface and is first measurable between one and two weeks after plucking at 32°C, but at 16°C the new hair is not measurable above the skin surface until two to three weeks after plucking. Apparently the environmental temperature does not affect the rate of hair growth but only the beginning processes (CHASE 1954) in the formation of the new hair.

Subcutaneous temperature

	at 16°C	at 32°C
The day after plucking	34.3 ± 0.2°C	38.6 ± 0.1°C
8 days after plucking	33.2 ± 0.1°C	38.4 ± 0.1°C
15 days after plucking	32.6 ± 0.2°C	38.1 ± 0.1°C
23 days after plucking	33.5 ± 0.2°C	37.3 ± 0.2°C
30 days after plucking	32.8 ± 0.2°C	38.4 ± 0.3°C
Mean	33.3°C	38.2°C
Unplucked controls after 7 days exposure	33.1 ± 0.2°C	37.7 ± 0.1°C

To determine the temperature of the uterine environment in which the first coat of hair of the guinea pigs is produced the rectal temperatures of twelve adult female animals in advanced stages of pregnancy were measured with the same thermometer used to calibrate the thermocouple. The mean rectal temperature was 39.1°C ± 0.1°C.

With low environmental temperatures there is apparently an adjustment of the animal's thermoregulatory mechanism to decrease the amount of heat lost through the skin. These temperatures are similar to those used by DANNEEL (1941) and ILJIN (1931) to show the effect of temperature on melanin formation in Himalayan rabbits.

By comparing the subcutaneous temperatures of the plucked and unplucked animals at the two experimental temperatures it will be apparent that in contrast to the situation in many mammals the hair does not insulate the guinea pig from the temperature of the environment.

Guinea pig coat color changes

The loci controlling hair pigment formation in the guinea pig dealt with in this study were *E*, *B*, *C*, *P*, and *F*. No agouti (*A*) or tortoise shell (e^p) animals were used. The *E* locus determines whether eumelanin or phaeomelanin is produced; animals carrying *E* being black, sepia, or brown, e^p giving the tortoise shell pattern (eumelanic and phaeomelanic areas in different parts of the coat) and *ee* being red, yellow, or cream. The combination *E-B-* produces a black or sepia animal, while *E-bb* causes brown melanin to be formed except in $c^a c^a$ albinos and in the combination *ppff* in both cases. In combination with *ee* the *B* locus determines only the color of the eyes and skin. The C series of alleles (*C*, c^k, c^d, c^r, c^a) roughly determines the intensity of the coat color in both eumelanic and phaeomelanic series; *C* producing black or red animals and $c^a c^a$ animals being albinos, with the other alleles causing intermediate amounts of pigment to be formed. In this allelic series the chief peculiarities in the relations between the amounts of pigment formed under the control of these genes occur in the two alleles c^d and c^r. In combination with *ee* the allele c^d produces a

TABLE 1

Mean reflectionmeter readings for all the genotypes studied. The mean per cent change is derived from percentage changes of individual animals and not from mean reflection meter readings

Genotype	Class	No. in class	Mean age (days)	Mean reflectionmeter readings								
				Amber			Green			Blue		
				Before exposure	After exposure	Mean % change ± S.E.	Before exposure	After exposure	Mean % change ± S.E.	Before exposure	After exposure	Mean % change ± S.E.
E-B-C-P-	16°C plucked	9	40.8	6.6	4.7	−25.5 ± 11.4	6.5	4.6	−25.8 ± 11.0*	5.4	4.2	−19.8 ± 11.2
	16°C control	10	44.2	6.0	6.7	+16.9 ± 12.4	5.8	6.1	+7.5 ± 8.4	5.1	5.4	+9.2 ± 11.0
	32°C plucked	9	44.2	6.0	5.5	−5.9 ± 6.4	5.6	5.0	−9.0 ± 6.9	5.1	4.7	−5.5 ± 5.0
	32°C control	10	45.0	5.8	5.7	+0.6 ± 6.1	5.5	5.3	−2.4 ± 5.4	4.8	4.8	−0.2 ± 5.8
E-B-$c^k c^r$P-	16°C plucked	3	43.0	6.6	4.3	−34.9 ± 2.5**	6.4	3.9	−38.9 ± 6.7*	5.1	3.7	−28.1 ± 4.3*
	16°C control	3	35.0	5.7	5.8	+3.0 ± 7.7	5.2	5.8	+13.5 ± 14.9	4.5	4.7	+5.6 ± 10.7
	32°C plucked	4	32.0	7.0	5.3	−23.4 ± 10.2	6.2	5.3	−13.3 ± 8.8	5.4	4.6	−17.1 ± 8.4
	32°C control	3	31.0	6.8	6.6	−2.9 ± 2.2	5.7	5.9	+3.1 ± 5.8	5.3	5.1	−3.3 ± 3.3
E-B-$c^d c^r$P-	16°C plucked	4	35.2	8.2	4.7	−41.0 ± 5.9**	7.5	4.3	−41.0 ± 6.5**	5.7	4.0	−29.1 ± 4.0**
	16°C control	3	45.0	7.7	6.2	−17.1 ± 13.1	6.7	6.2	−8.9 ± 8.6	5.4	5.3	−2.6 ± 8.7
	32°C plucked	3	41.7	7.1	5.1	−28.8 ± 3.6*	6.2	4.6	−25.7 ± 5.2*	5.1	4.3	−16.3 ± 8.3
	32°C control	3	44.7	6.6	6.8	+2.7 ± 7.9	6.4	6.0	−6.4 ± 8.8	5.3	5.1	−3.6 ± 2.1
E-B-$c^d c^a$P-	16°C plucked	8	51.6	14.9	6.0	[z]−59.3 ± 3.2**	13.6	6.1	[y]−54.1 ± 5.3**	10.5	5.3	[z]−47.5 ± 6.2**
	16°C control	8	57.1	14.2	11.6	−14.5 ± 8.8	12.9	9.9	−19.8 ± 7.2*	9.0	7.7	−9.8 ± 10.1
	32°C plucked	5	33.4	15.0	11.2	−25.7 ± 8.4*	15.1	10.4	−26.6 ± 12.2	10.0	8.7	−11.5 ± 8.8
	32°C control	4	32.3	15.4	14.7	−4.7 ± 4.6	13.7	13.6	−1.2 ± 4.8	9.9	9.3	−6.7 ± 3.2
E-B-$c^r c^r$P-	16°C plucked	11	38.5	6.9	5.2	[z]−29.5 ± 2.6**	6.5	4.4	[z]−32.3 ± 3.7**	5.2	4.1	−21.8 ± 3.7**
	16°C control	11	34.4	6.3	6.2	−0.3 ± 8.2	6.3	5.7	−8.5 ± 7.0	5.4	5.2	−1.3 ± 7.0
	32°C plucked	11	46.2	6.8	5.9	−11.3 ± 4.7*	6.3	5.5	−11.7 ± 4.7*	5.4	4.8	−9.5 ± 4.5
	32°C control	11	36.6	6.9	5.9	−13.1 ± 3.5**	6.4	5.6	−12.6 ± 3.1**	5.5	4.8	−13.6 ± 1.8**
E-B-$c^r c^a$P-	16°C plucked	13	44.8	11.1	6.6	−39.3 ± 5.5**	10.2	6.2	−35.6 ± 6.3**	7.6	5.3	−28.5 ± 5.5**
	16°C control	13	44.3	11.3	8.3	−25.1 ± 4.3**	10.6	7.6	−25.6 ± 4.1**	7.8	6.1	−20.1 ± 4.3**

E-B-c^{t}c^{a}P-	32°C plucked	12	40.9	11.4	7.3	−31.2 ± 5.3**	10.3	7.0	−27.0 ± 5.5**	7.9	6.5	−15.2 ± 7.2
	32°C control	14	37.9	12.7	10.1	−18.1 ± 5.1**	11.9	9.8	−15.0 ± 4.3**	9.6	7.2	−18.4 ± 5.9**
E-c^{a}c^{a}P-	16°C plucked	9	50.3	104.3	78.1	[z]−25.2 ± 2.3**	98.8	71.5	[z]−27.7 ± 2.3**	82.3	58.3	[z]−29.2 ± 2.8**
ee/pp-c^{a}c^a	16°C plucked	7	37.4	104.5	100.7	−3.5 ± 2.0	99.9	95.6	−4.1 ± 2.4	85.9	81.1	−5.3 ± 2.9
	16°C control	15	47.4	104.4	103.4	−0.9 ± 1.3	100.0	98.7	−1.2 ± 1.3	84.5	80.7	−4.2 ± 2.1
	32°C plucked	16	38.9	106.8	99.9	−6.4 ± 1.0**	102.8	96.8	−5.8 ± 0.7**	88.1	82.1	−7.1 ± 1.0**
	32°C control	16	34.4	104.0	103.0	−1.0 ± 1.2	99.2	99.3	+0.1 ± 1.4	84.5	82.4	−2.1 ± 1.5
E-B-C-pp-F-	16°C plucked	12	37.6	34.4	46.8	+43.9 ± 10.6**	29.9	42.4	+49.3 ± 10.8**	22.4	31.6	+54.1 ± 14.5**
	16°C control	12	37.6	38.0	41.1	+9.5 ± 2.8**	33.7	37.0	+13.3 ± 6.0*	25.0	26.2	+8.3 ± 6.0
	32°C plucked	12	37.2	38.0	51.8	+44.5 ± 9.6**	34.2	46.3	+43.3 ± 9.3**	26.1	36.7	+51.4 ± 11.5**
	32°C control	13	42.7	35.9	41.5	+17.4 ± 4.8**	31.2	35.5	+15.0 ± 4.3**	23.4	25.1	+9.3 ± 6.3
E-B-c^{t}c^{t}ppF-	16°C plucked	7	43.9	64.4	55.6	[y]−12.9 ± 4.8*	59.6	50.4	[y]−14.8 ± 4.5*	49.8	39.9	[y]−19.5 ± 4.8**
	16°C control	6	31.5	63.4	60.5	−3.5 ± 3.1	57.9	54.5	−4.3 ± 4.6	48.0	43.1	−8.2 ± 5.6
	32°C plucked	8	33.1	61.9	61.2	−1.0 ± 2.2	56.8	56.3	−0.6 ± 2.8	45.4	43.7	−3.3 ± 3.5
	32°C control	6	43.0	58.2	60.0	+3.4 ± 3.9	52.8	52.1	−0.8 ± 4.2	42.5	40.8	−3.8 ± 6.3
E-bbC-Pp	16°C plucked	12	44.9	13.1	11.7	−8.1 ± 7.5	11.3	10.0	−8.3 ± 9.0	8.8	7.4	−12.3 ± 8.6
	16°C control	11	43.4	14.4	12.4	−10.1 ± 11.1	12.5	10.9	−6.9 ± 11.8	8.8	7.8	−7.0 ± 10.8
	32°C plucked	12	37.7	13.7	10.0	−21.0 ± 8.6*	11.8	8.8	−20.9 ± 6.7**	7.9	6.5	−13.4 ± 8.5
	32°C control	12	43.1	11.6	12.5	+7.0 ± 7.9	10.3	10.7	+9.2 ± 9.2	7.3	8.2	+19.8 ± 14.6
E-bbc^{t}c^{a}P-	32°C plucked	5	38.2	14.7	11.4	−21.8 ± 7.3*	13.6	10.7	−22.5 ± 6.3*	9.9	7.9	−19.8 ± 7.0*
E-bbC-ppF-	16°C plucked	16	42.3	41.4	41.9	+2.1 ± 3.8	35.1	35.0	[y]+1.3 ± 4.1	22.3	22.8	[y]+3.2 ± 5.1
	16°C control	15	36.1	34.9	37.9	+11.2 ± 6.1	30.1	31.7	+7.1 ± 5.3	18.6	20.6	+12.7 ± 8.9
	32°C plucked	16	37.1	40.5	45.0	+13.8 ± 4.4**	34.3	39.1	+16.5 ± 4.6**	22.3	26.3	+19.5 ± 4.6**
	32°C control	16	38.4	40.0	41.5	+4.7 ± 3.0	35.3	34.5	−1.7 ± 4.3	22.2	21.8	−0.3 ± 3.7
E-bbc^{t}c^{t}ppF-	16°C plucked	5	27.1	68.0	66.2	−1.4 ± 3.7	61.9	59.4	−4.0 ± 3.0	48.2	44.6	−7.0 ± 5.2
	16°C control	4	39.0	68.9	70.4	+2.6 ± 3.1	62.6	63.3	+1.0 ± 2.0	46.8	48.9	+5.5 ± 5.6
E-C-ppff	16°C plucked	12	43.8	85.5	87.8	[y]+2.9 ± 1.8	77.2	80.9	[y]+5.4 ± 2.2*	56.9	64.3	+13.6 ± 3.5**
	16°C control	12	37.2	85.8	89.5	+4.7 ± 2.0*	78.0	81.6	+5.0 ± 2.2*	58.0	61.3	+6.8 ± 3.1
	32°C plucked	11	29.0	83.4	92.0	+10.9 ± 3.0**	73.9	84.4	+14.6 ± 2.5**	53.2	64.5	+21.9 ± 3.4**
	32°C control	11	34.2	88.4	91.9	+4.2 ± 1.7*	80.6	83.5	+4.6 ± 2.2	61.5	63.7	+4.1 ± 2.5

TABLE 1—*Continued*

Genotype	Class	No. in class	Mean age (days)	Mean reflectionmeter readings								
				Amber			Green			Blue		
				Before exposure	After exposure	Mean % change ± S.E.	Before exposure	After exposure	Mean % change ± S.E.	Before exposure	After exposure	Mean % change ± S.E.
eeC-ff	16°C plucked	6	51.2	61.9	67.7	[y]+10.2 ± 4.5	49.6	58.0	+18.7 ± 8.4	27.3	36.5	+37.7 ± 13.0*
	16°C control	4	33.3	62.4	63.2	+1.6 ± 3.8	52.7	51.3	−2.1 ± 5.1	28.1	27.6	+0.2 ± 9.5
	32°C plucked	4	38.3	56.0	72.5	+29.5 ± 5.7*	45.2	62.0	+37.6 ± 6.8*	20.9	36.2	+49.0 ± 19.0
	32°C control	3	39.3	64.2	64.5	+0.5 ± 5.0	53.4	53.1	−0.2 ± 6.0	28.4	28.6	+4.7 ± 15.2
eeB-C-P-F-	16°C plucked	12	49.9	39.3	44.4	+17.4 ± 7.3*	30.7	35.1	+19.5 ± 8.2*	14.1	16.0	+23.3 ± 11.6
	16°C control	12	58.2	37.3	40.2	+9.7 ± 4.4	29.0	30.6	+8.0 ± 5.3	12.5	13.6	+16.2 ± 10.2
	32°C plucked	14	39.5	35.3	42.4	+23.0 ± 5.7**	26.2	31.8	+24.9 ± 5.9**	12.1	14.4	+21.1 ± 5.6**
	32°C control	13	38.4	33.6	35.0	+5.7 ± 3.3	25.3	26.5	+6.4 ± 4.0	12.2	11.6	−1.9 ± 4.1
ee-bbC-P-F-	16°C plucked	8	41.1	39.3	44.2	+14.5 ± 5.0*	29.5	33.7	+16.8 ± 5.8*	13.7	17.9	+34.0 ± 14.0*
	16°C control	8	36.0	37.8	41.6	+13.0 ± 7.1	29.4	32.1	+12.2 ± 7.3	13.4	16.0	+28.1 ± 21.1
	32°C plucked	7	37.6	39.3	41.9	+7.3 ± 4.7	30.1	31.9	+6.3 ± 4.4	13.9	14.1	+3.5 ± 7.9
	32°C control	8	42.0	37.6	41.1	+10.5 ± 6.4	27.7	32.4	+18.2 ± 7.0*	12.6	14.2	+15.2 ± 9.2
eeC-pp-F-	16°C plucked	5	48.2	37.8	43.8	+18.8 ± 9.7	30.5	34.5	+16.9 ± 11.9	12.8	15.0	+20.3 ± 11.2
	16°C control	5	39.0	43.0	42.5	−1.2 ± 1.4	33.7	33.3	+0.2 ± 3.1	15.6	15.0	−3.4 ± 3.3
	32°C plucked	5	53.2	43.8	46.3	+11.1 ± 6.3	33.6	35.5	+8.3 ± 7.9	15.2	16.1	+12.9 ± 14.2
	32°C control	5	39.6	34.6	36.5	+6.7 ± 5.8	27.1	27.5	+3.6 ± 6.5	12.0	11.9	−0.2 ± 4.8
eec^{k}c^{k}P-F-	16°C plucked	6	42.2	56.5	54.2	−4.2 ± 1.6*	46.8	44.1	−5.5 ± 3.6	22.9	22.3	−2.6 ± 3.5
	16°C control	6	28.3	54.7	56.1	+2.8 ± 2.1	44.5	45.7	+2.9 ± 2.2	21.8	23.7	+9.1 ± 3.2*
	32°C plucked	6	43.0	59.2	56.4	−4.6 ± 3.0	49.2	46.0	−6.0 ± 3.8	25.6	23.8	−5.7 ± 6.1
	32°C control	5	35.0	56.3	54.1	−3.6 ± 3.3	45.7	42.9	−5.7 ± 3.6	23.3	21.0	−9.2 ± 5.1
eec^{k}c^{d}P-F-	16°C plucked	4	55.3	52.4	48.7	−6.5 ± 4.2	40.8	37.5	−7.9 ± 2.7	21.0	17.7	−14.0 ± 7.0
	16°C control	4	45.8	52.3	50.5	−3.4 ± 3.3	41.9	40.9	−2.3 ± 5.7	19.5	19.0	−0.4 ± 10.8
	32°C plucked	3	51.3	54.7	50.8	−6.6 ± 5.4	43.4	40.6	−5.7 ± 5.9	22.8	19.9	−8.9 ± 11.2
	32°C control	3	54.7	54.7	57.4	+5.0 ± 1.2	44.2	45.9	+3.8 ± 1.4	22.2	23.8	+7.8 ± 14.5

eec^{k}*c*^{a}*P-F-*	16°C plucked	8	37.8	70.7	65.3	−7.6 ± 2.4*	60.2	53.3	^{y}−11.4 ± 4.4*	36.6	35.8	−0.8 ± 8.6
	16°C control	9	37.2	72.8	72.3	−0.5 ± 2.6	63.0	63.3	+0.8 ± 2.2	38.2	40.7	+8.0 ± 5.7
	32°C plucked	9	38.9	72.2	70.1	−2.9 ± 2.1	61.0	61.5	+1.0 ± 2.7	38.1	38.5	+1.5 ± 3.9
	32°C control	9	40.9	77.7	74.5	−4.0 ± 1.9	67.4	64.2	−4.8 ± 1.9*	42.0	39.5	−5.6 ± 2.8
eec^{d}*c*^{d}*P-F-*	16°C plucked	7	35.1	58.9	49.0	^{z}−17.0 ± 1.7**	46.0	39.0	^{y}−14.9 ± 2.7**	25.8	20.1	−20.9 ± 4.9**
	16°C control	8	30.8	58.6	59.4	+1.8 ± 2.7	47.7	48.5	+2.4 ± 2.8	24.7	26.3	+9.0 ± 5.4
	32°C plucked	8	38.3	62.6	60.3	−3.8 ± 1.5*	51.9	50.2	−2.8 ± 4.2	29.1	27.3	−5.0 ± 6.5
	32°C control	7	35.6	62.1	58.3	−5.9 ± 2.3*	51.7	47.9	−7.1 ± 3.5	29.8	23.8	−18.0 ± 6.7*
eec^{d}*c*^{d}*ppF-*	16°C plucked	7	41.7	63.4	56.7	^{z}−10.3 ± 2.2**	53.2	47.1	^{z}−11.2 ± 2.7**	29.2	24.1	^{y}−16.5 ± 4.7*
	16°C control	7	52.6	61.6	60.3	−1.6 ± 2.8	50.4	51.1	+2.8 ± 4.9	28.0	27.7	+1.8 ± 8.2
	32°C plucked	7	39.7	59.9	61.3	+2.8 ± 3.2	48.1	49.9	+4.7 ± 4.5	26.4	27.1	+4.6 ± 6.8
	32°C control	7	32.0	58.3	57.3	−1.5 ± 2.0	47.7	46.9	−0.9 ± 3.2	24.2	23.2	−1.6 ± 5.8
eec^{d}*c*^{r}*P-F-*	16°C plucked	4	38.5	75.5	59.5	^{y}−21.3 ± 5.7*	64.4	49.5	^{y}−23.5 ± 4.2*	39.9	27.6	^{y}−31.5 ± 4.0**
	16°C control	4	30.5	73.8	71.1	−3.3 ± 4.6	64.1	59.7	−6.7 ± 2.7	41.7	34.7	−15.9 ± 5.2
	32°C plucked	6	38.8	71.0	69.0	−2.5 ± 3.3	61.1	58.7	−3.5 ± 4.2	39.7	38.1	−2.9 ± 7.3
	32°C control	5	35.8	73.1	73.9	+1.3 ± 1.8	64.0	62.1	−2.3 ± 3.7	40.9	40.6	−0.0 ± 7.3
eec^{d}*c*^{a}*P-F-*	16°C plucked	7	41.0	74.8	61.3	^{z}−17.4 ± 3.9**	65.7	51.3	^{z}−21.1 ± 4.2**	40.4	28.5	^{z}−29.0 ± 3.6**
	16°C control	8	53.1	81.5	75.8	−6.7 ± 3.1	72.8	66.7	−8.5 ± 2.9*	49.0	44.2	−9.9 ± 5.1
	32°C plucked	10	43.4	78.0	75.2	−3.2 ± 1.5	67.8	65.3	−3.1 ± 2.2	43.5	42.1	−1.7 ± 3.1
	32°C control	8	31.0	76.0	72.6	−4.4 ± 2.6	65.9	62.4	−5.3 ± 2.3	41.6	37.0	−10.9 ± 3.2*
eec^{d}*c*^{a}*ppF-*	16°C plucked	5	43.4	85.6	72.8	−14.7 ± 4.8*	76.1	62.1	^{y}−18.2 ± 4.6*	53.1	39.7	−23.4 ± 9.6
	16°C control	4	47.0	80.0	72.3	−9.5 ± 0.8**	69.8	62.4	−10.8 ± 1.1**	45.5	38.3	−16.1 ± 5.3
	32°C plucked	6	35.5	79.6	76.3	−3.8 ± 3.3	70.2	68.1	−2.6 ± 4.0	46.9	44.2	−5.3 ± 6.3
	32°C control	3	30.0	78.5	76.8	−1.9 ± 4.4	68.8	64.4	−6.3 ± 4.4	46.7	39.6	−14.0 ± 9.4
eec^{r}*c*^{a}*P-*	16°C plucked	3	30.7	106.0	88.4	^{z}−16.7 ± 1.2**	102.4	83.5	^{z}−18.4 ± 1.7**	85.7	67.9	^{z}−20.7 ± 3.6*
	16°C control	3	28.7	105.6	108.4	+2.6 ± 1.9	101.5	102.8	+1.2 ± 2.4	84.9	82.5	−2.9 ± 1.6
	32°C plucked	4	33.3	107.4	102.7	−4.4 ± 1.5	103.8	99.7	−4.0 ± 1.9	89.3	83.5	−6.6 ± 2.0*
	32°C control	4	32.8	101.4	106.5	+4.9 ± 2.1	95.4	102.3	+7.7 ± 3.7	79.8	83.8	+5.9 ± 4.6

* Significantly different from 0% change—P = .01–.05.
** Significantly different from 0% change—P = <.01.
^{y} = Significantly different from the change in 32°C Plucked—P = .01–.05.
^{z} = Significantly different from the change in 32°C Plucked—P = <.01.

cream-colored animal, while *eec*^{r}- has no pigment in the hair at all but has dark red eyes and in combination with the pink eye dilution factor (*p*) cannot be differentiated from an albino (*c*^{a}*c*^{a}) guinea pig except by a breeding test. In combination with *E*- the allele *c*^{r} forms considerably more sepia or brown pigment than does *c*^{d} in contrast to the situation in the phaeomelanic series. The pink eye dilution factor (*p*) greatly reduces the amount of eumelanin formed although it has no effect on the coat color in *ee* animals except to prevent sootiness from developing with age and/or cold. The recessive allele (*f*) at the *F* locus when homozygous in yellows (*ee*) and in *E-pp* genotypes decreases the amount of pigment formed very markedly. The effects on guinea pig coat color of the alleles at these loci, the changes in coat color intensity with age in the various genotypes (WRIGHT 1949), and the changes in coat color with different environmental temperatures (table 1) will be discussed below.

The percentages of pigmentation in adults cited in WRIGHT's data are relative to the amounts in intense blacks, browns, or reds at birth as determined by colorimetry of pigment extracted from weighed hair samples in conjunction with visual grades, standardized by this process. (HEIDENTHAL 1940, RUSSELL 1939, WRIGHT and BRADDOCK 1949, WRIGHT 1949)

Dark sepias.—These are genotypes with the genes *E-B-P-* and various combinations of the alleles of the C-series. *E-B-C-P-*, intense black, fades slightly from birth (100.0%) to adulthood (95.6%) while *E-B-c*^{d}*c*^{r}*P-* does not change appreciably (92.8% to 92.3%). The dark sepia genotypes *E-B-c*^{k}*c*^{r}*P-* and *E-B-c*^{r}*c*^{r}*P-* darken somewhat with age (71.4% to 79.4% and 83.1% to 86.1%) while very pronounced darkening between birth and adulthood occurs in *E-B-c*^{d}*c*^{a}*P-* and *E-B-c*^{r}*c*^{a}*P-* (38.2% to 54.5% and 40.6% to 70.8%). There is pronounced darkening of the ears, feet, and nose (completely colorless at birth) and occasionally a slight darkening of the coat in albinos of the genotype *E-B-c*^{a}*c*^{a}*P-*. (WRIGHT 1949)

In the present investigation all groups of the genotype *E-B-C-P-* except the 16°C control group had a tendency to become darker, while the latter tended to fade. However, the variability in all four categories was so great that none of the changes were significant at the .05 level except in the 16°C plucked group and this only with the green filter (a darkening of about 25%). It is concluded that there was no appreciable effect of the experimental temperatures on pigment formation in this case. Different conclusions may be drawn about the genotypes *E-B-c*^{k}*c*^{r}*P-* and *E-B-c*^{d}*c*^{r}*P-*. There was significant darkening of the 16°C plucked group in both cases but enough darkening in the 32°C plucked group (significant at the .05 level in *c*^{d}*c*^{r} but not in *c*^{k}*c*^{r}) to prevent any significant effect at the .05 level of the temperature difference.

The data obtained on *E-B-c*^{d}*c*^{a}*P-* and *E-B-c*^{r}*c*^{r}*P-* show a pronounced effect of the 16°C temperature, plucked *E-B-c*^{d}*c*^{a}*P-* animals darkening 59% in the amber range at 16°C but only 26% at 32°C, plucked *E-B-c*^{r}*c*^{r}*P-* darkening 29.5% and 11% respectively in the same region of the spectrum. Each of these changes is significant (.01 level) showing that there is not only a darkening due to the low temperature but also due to other factors connected with age unless the slight drop in skin temperature from 39.1°C *in utero* to an average of 38.2°C at 32°C room temperature is sufficient to induce increased melanin production.

The genotype *E-B-$c^r c^a$P-* shows a pronounced darkening in all groups but while greatest in the 16°C plucked category the difference from the 32°C plucked group has no statistical significance. It does not seem likely, however, that there is any qualitative difference in the reactions of $c^r c^a$ and the two homozygotes, $c^r c^r$ and $c^a c^a$, both of which show a significant temperature effect. Another peculiarity of this genotype is the highly significant (.01 level) darkening of the controls also found, however, in *E-B-$c^r c^r$P-* in the 32°C control group and in *E-B-$c^d c^a$P-* at the .05 level (green filter) in the 16°C controls. It appears that there is a difference in either the threshold of response or in the responsiveness to other factors than cold between the c^r and c^d genotypes.

In albinos of genotype *E-$c^a c^a$P-* the 16°C environmental temperature had a very striking effect, the rumps of plucked animals becoming quite dark. This observation also confirms the results of ILJIN (1927) and SCHULTZ (1922) showing pigment formation with cold in albino guinea pigs.

Although it is usually possible to differentiate between *E-$c^a c^a$P-* and *E-$c^a c^a$pp*, *ee$c^a c^a$P-*, or *ee$c^a c^a$pp* albinos by the sooty nose, ears, and feet of the first, this darkening does not occur uniformly or at the same age in all *E-$c^a c^a$P-* albinos. Because some of the light-eared albino animals grew new dark hair when plucked and exposed to 16°C, as the assignment of genotypes to the albinos was on a very tentative basis, and since in all experimental categories except 16°C plucked, all animals—plucked or unplucked—darkened only very slightly if at all (less than 20%), it was decided to test, by independent evidence, the hypothesis that only *E-$c^a c^a$P-* animals produced eumelanic pigment after being plucked and exposed to 16°C.

The percentage of yellow (*ee*) and pink-eyed (*pp*), sepia and brown and tortoise shell (*E-P-*, *e^p-P-*) litter mates of each of the albino animals which darkened more more than 20% and those which darkened less than 20% were calculated and the mean percentage obtained. The percentage of yellow and/or pink-eyed (*ee/pp*) littermates of albinos which darkened more than 20% was 34.1% ± 10.2%, whereas the percentage of these genotypes among littermates of albinos darkening less than 20% was 59.4% ± 14.5%. The *ee/pp* percentage among littermates of the unplucked albinos kept at 16°C was only 25.9% ± 9.6% but all the albinos in this category darkened less than 20%. The yellow/pinkeye percentage among littermates of plucked albinos exposed to 32°C was 41.4% ± 6.2%, of unplucked albinos kept at 32°C 34.2% ± 7.7%. All of the albinos in the last two categories darkened less than 20%. These data are rather strong evidence for the hypothesis that only *E-$c^a c^a$P-* albinos have the capacity to respond to low temperature by producing eumelanic pigment.

The darkening of the albinos in the *ee/pp $c^a c^a$* plucked lot and in the animals in the 32°C plucked category was not visually perceptible and may have been due to dirt.

On comparing these results with the changes described by WRIGHT (1949) as occurring in *E-B-P-* genotypes between birth and adulthood it appears that much of the latter is a temperature effect but that there is darkening that occurs with only a 1°C drop in skin temperature, especially in $c^r c^a$. but to some extent in others and that may be due to other factors. There seems also to be some fading of intense black subsequent to the second pelage.

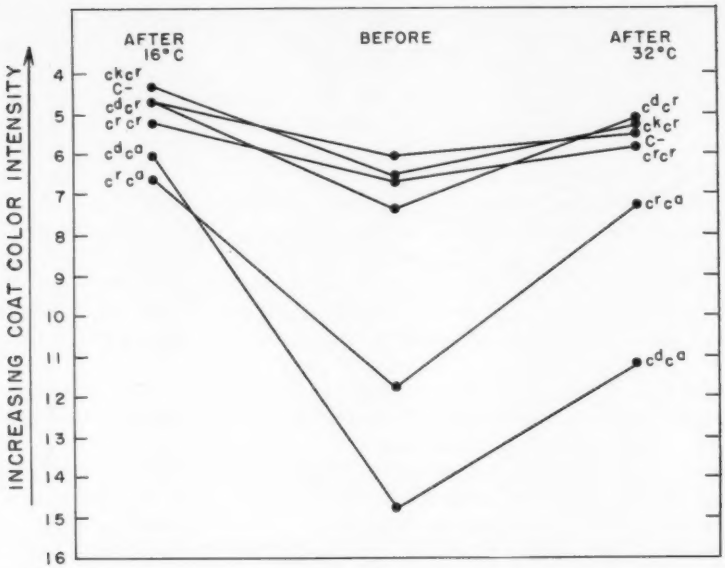

FIGURE 1. Graphical representation of the changes in the amber reflection meter readings of dark sepia (*E-B-P-*) genotypes. Ordinate represents actual readings as given in table 1. The points in the "Before" category on the abscissa represent the weighted means of all "Before" readings (amber) for each genotype given in table 1. The points in the two "After" categories represent the mean readings (table 1) after plucking and exposure to the experimental temperatures.

Pale sepias.—Of the *E-B-ppF-* genotypes only two will be considered as not enough animals of the other genotypes were available. All pale sepia genotypes except $c^r c^r$ and $c^r c^a$ fade considerably with age, *E-B-C-ppF-* fading from 17% to 7.5%. *E-B-*$c^r c^r$ *ppF-* darkens slightly (1.9% to 2.9%) between birth and adulthood. (WRIGHT 1949) Genotypes of the kind *E-B-ppff* are white except *E-B-C-ppff* which is cream-colored. These genotypes will be considered separately.

The above mentioned age effects are paralleled in the present experiment. Plucked animals of genotype *E-B-C-ppF-* become about 43% to 54% lighter at both 16°C and 32°C, while plucked *E-B-*$c^r c^r$ *ppF-* animals darken about 15% with the second pelage but only at 16°C.

There are no temperature effects on pigment production in *E-B-C-ppF-* that are significant at the .05 level. The significant fading in the control categories may be due to replacement of old hair during the experimental period.

E-B-$c^r c^r$ *ppF-* shows a significant temperature effect. Apparently the major part, if not all, of the age effect of this genotype is due to the drop in skin temperature while the pronounced fading of all the more intense *E-B-ppF-* genotypes in adults is not a temperature effect, as judged from the absence of such effect in *E-B-C-ppF-*.

Dark browns.—All combinations of the *C* alleles with *E-bbP-* fade from birth except *E-bbc*$^d c^a$*P-* and *E-bbc*$^r c^a$*P-* which darken. *E-bbC-P-* fades from 100% to 73.5%, while

E-bbc^{r}c^{a}P- darkens from a birth average of 59.5% to 70.9% as an adult. (WRIGHT 1949)

In the present investigation the only *C*-browns used were of genotype *E-bbC-Pp* as *E-bbCCPP* show a variable "dinginess" (WRIGHT 1947) which would confuse the reflection meter readings.

E-bbC-P- shows no significant temperature effects in the animals used which were an average forty days old at the beginning of the experimental periods. The apparently significant darkening in the plucked animals of the 32°C category seems to indicate a darkening in the second pelage not due to temperature which contrasts with the marked lightening found by WRIGHT (1949) in adults. The changes in browns clearly need more study.

E-bbc^{r}c^{a}P- shows a significant amount of darkening (about 20%) in animals plucked and exposed to 32°C. This is in agreement with the age effect. As only very few animals in the other categories were available no conclusions as to the relation of temperature to the age effect can be drawn in this genotype.

Pale browns.—All pale brown genotypes (*E-bbppF-*) fade with age except *c^{r}c^r* and *c^{r}c^a*. *E-bbC-ppF-* has 27.8% as much pigment at birth as *E-bbC-P-* but as an adult only 18.9% as much. *E-bbc^{r}c^{r}ppF-* darkens slightly with age (4.0% to 5.3%). (WRIGHT 1949)

E-bbppff genotypes are similar to *E-B-ppff* genotypes in being white except the cream-colored *E-bbC-ppff* animals.

E-bbC-ppF- animals give complex results. There is an apparently significant temperature effect but this depends on significant lightening (.01 level) in the 32°C plucked group associated with no appreciable change in the 16°C plucked category. This can only be interpreted as a genuine temperature effect by the hypothesis that there is a lightening of 16% not due to temperature during this period (as shown by the 32°C plucked group), balanced by darkening due to cold of almost the same amount in the 16°C plucked group. The fading effect appears to become more pronounced in adults.

The slight darkening effect observed with age in *E-bbc^{r}c^{r}ppF-* by WRIGHT (1949) is paralleled only by an utterly insignificant tendency in this direction in the present data. As too few animals were available no temperature comparisons could be made.

Anomalous creams.—As mentioned above this category includes animals of genotypes *E-bbC-ppff* and *E-B-C-ppff* which are cream-colored (although a faint brownish tinge is observable in some animals). They fade from a birth average of 6.3% to an adult average of 3% (WRIGHT 1949).

The age effect of fading is paralleled in the present data.

There is also a significant temperature effect opposite to the age effect. At 32°C plucked animals show a greater degree of fading in the second pelage than at 16°C. As in *E-bbC-ppF-* this may be due to a slowdown due to cold of the normal decrease in pigment production in *E-C-ppff* with age which is equivalent to a darkening due to cold in other genotypes.

Fading yellows.—All *eeff* genotypes which are not white at birth fade with age. These include *C-*, *c^{k}c^k*, *c^{k}c^d*, *c^{d}c^d*, *c^{k}c^a*, *c^{d}c^a*. *eeC-ff* fades from 35.4% yellow pigment at birth to 10.8% as an adult. (WRIGHT 1949)

This age effect is paralleled by the present results.

There is an apparently significant temperature effect (a decreased amount of fading at 16°C as compared to 32°C but only with one filter and only at the .05 level). If not due to an extreme sampling error, it may be the result of a tendency toward increased rate of pigment production induced by low skin temperature that partially compensates for fading due to other causes as suggested for other genotypes above.

In comparing the percentage amount of fading at 32°C in plucked animals of this genotype (30 to 49%) with that in *eeC-F-* (4 to 25%) it may be seen that the *ff* yellows have faded more on the average by two and one-half months of age than the *F*-yellows but the difference is less pronounced than found in the adults by WRIGHT (1949).

Stable yellows.—These are *eeF-* genotypes which produce a larger amount of phaeomelanic pigment than the comparable *eeff* genotypes and which either fade considerably less with age than the latter or darken slightly in some cases. Genotypes which fade with age include *eeC-F-* (100% at birth to 76% as adults), eec^kc^kF- (36% to 30%), and eec^kc^aF- (17.5% to 12%). Genotypes which darken with age include eec^kc^dF- (33% to 40% with inadequate numbers), eec^dc^dF- (36% to 37%), eec^dc^rF- (12% to 16%), and eec^dc^aF- (14% to 16.5%). eec^rc^r and eec^rc^a animals are white with dark red and light red eyes respectively. These never show any yellow pigment but as with *B-P-* yellows, old animals will sometimes show a eumelanic sootiness in their coat. (WRIGHT 1949) The genes *B*, *b*, *P*, *p*, have no effect on the coat color in yellow animals; they only affect the eye color. *pp* probably prevents the appearance of sootiness in the coat of yellow guinea pigs.

The age effect of fading is paralleled in all three *eeC-F-* genotypes tested in the present experiments. There are no differences among them. There are no significant temperature effects on pigment formation in these three genotypes. The figures in table 1 are all based on non-sooty animals. It is thus indicated that the normal fading with age is not a temperature effect. In addition there were two *eeB-C-P-F-* animals which were plucked and exposed to 16°C which formed eumelanic pigment in the yellow hairs. One of these animals showed eumelanic subterminal bands in the otherwise red hairs. The percentage of darkening in this animal was amber: −45.8%, green: −47.7%, blue: −15.5%. The other animal had black tips on the newly grown hair. The percentage change in this animal was amber: +8.4%, green: −14.9%, blue: −7.1%. It may be assumed that in the former animal the eumelanin was deposited in hairs which were already growing at the time of plucking but were not sufficiently long to be plucked. The sudden drop in skin temperature to 33.3°C apparently had the effect of temporarily decreasing the rate or efficiency of the yellow pigment forming process relative to the eumelanin forming process and thus allowed eumelanin to be formed until the normal competitive advantage of the phaeomelanic pigment forming process (in *ee* animals) was restored. This is additional evidence that both the eumelanic- and phaeomelanic pigment forming processes coexist in normally yellow guinea pigs. ILJIN (1927) and ENGELSMEIER (1937) reported that the width of the pigmented band in normally white hairs of Himalayan rabbits was correlated with the length of exposure to low temperature. In the guinea pig this is obviously not the case since the hair has no insulating effect. Genetic factors may make a difference in the threshold at which eumelanin may be formed in normally yellow guinea pigs (cf. IBSEN 1932).

eec^{k}c^{k}P-F- animals did not show the age effect of fading. There was no appreciable change (less than 10%) although what change there was, was in the direction of darkening. There was no temperature effect.

eec^{k}c^{d}P-F- darkened to about the same extent in percentage as *eec^{k}c^{k}P-F-*. Again there was no differential temperature effect. One animal, plucked and exposed to 16°C, became sooty and was not included in the table. Its percentage change: amber −44.6%, green −49.5%, blue −32.7%.

eec^{k}c^{a}P-F- also darkened about the same number of percentage points as the other two *c^k* genotypes. This result is in the opposite direction to the age effect. There is indeed an apparently significant darkening in the 16°C plucked group shown only with the green filter. Comparison of all of the figures indicates that there is no real difference from the situation in the *c^{k}c^k* and *c^{k}c^d* genotypes. Two animals (not included in the table) out of ten plucked and exposed to 16°C produced eumelanin in the new hair. One produced black hairs. It changed −52.6% in the amber, −46.8% in the green, and −41.1% in the blue region of the spectrum. The other animal had dark tips on the new hairs. It changed −11.0% in the amber, −15.8% in the green, and −13.8% in the blue region of the spectrum.

There was no appreciable difference in the amount or direction of change in the coat color between *eec^{d}c^{d}P-F-* and *eec^{d}c^{d}ppF-* guinea pigs.

There is a pronounced effect of low temperature on the amount of phaeomelanin produced in these genotypes. An environmental temperature of 16°C increases pigment formation over the amount produced at 32°C. The usual darkening in adults in these genotypes (WRIGHT 1949) thus appears to be a temperature effect.

The darkening is wholly due to the formation of phaeomelanin as the one *eec^{d}c^{d}P-F-* animal which had eumelanic subterminal bands in the yellow hairs was not included among the seven animals in the 16°C plucked group for which data are reported in the table. The percentages of darkening for this animal were: amber −43.3%, green −39.9%, blue −27.3%. These figures may be compared with the data for the other *eec^{d}c^{d}P-F-* animals which were plucked and exposed to 16°C: amber −17%, green −15%, blue −21%.

The results for *eec^{d}c^{r}P-F-*, *eec^{d}c^{a}P-F-*, and *eec^{d}c^{a}ppF-* parallel the aging effect of darkening. The percentage darkening in all three genotypes is about the same as in the genotypes just discussed and the effect of 16°C in increasing the formation of phaeomelanin is also similar. Animals which become sooty on exposure to 16°C were not included in the tabulated data. There was one *eec^{d}c^{r}P-F-* animal (in addition to the four reported in the table) which became sooty. It changed: amber −42.8%, green −42.0%, blue −47.7%. One *eec^{d}c^{a}P-F-* guinea pig had black-tipped yellow hairs. Its percentage change (amber −40.8%, green −52.1%, blue −48.9%) was not included in the seven animals whose data may be found in the table.

eec^{r}c^{a}P- showed a pronounced temperature effect, darkening about 17% after plucking and exposure to 16°C. This was wholly due to the formation of eumelanin and not to phaeomelanin.

It should be remarked that none of the yellow animals of any genotype which were plucked and exposed to 32°C became sooty. Thus it seems clear that the appearance of sootiness in a yellow animal requires a low skin temperature. The low frequency of sootiness even at low temperatures indicates that there are other necessary factors.

Where fading of *F*-yellows occurs with age (i.e. with *C*-), it appears that this is not due to temperature. On the other hand both the intensification of yellow in c^dc^d, c^dc^r, and c^dc^a genotypes and the sootiness in *B-P-* yellows and whites is probably wholly due to lowered skin temperature.

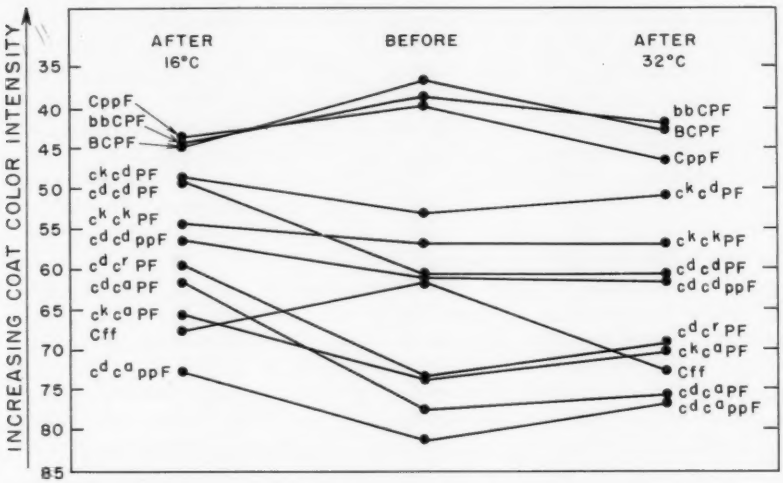

FIGURE 2. Graphical representation of the changes in the amber reflection meter readings of yellow (*ee*) genotypes. Ordinate and abscissa as in figure 1.

SUMMARY

1. The effects of environmental temperatures of 16°C and 32°C on coat color in different guinea pig genotypes were investigated by measuring the coat color of young guinea pigs (age about 1½ months) with a Photovolt photoelectric reflection meter, Model 610, before plucking the rump hair and again after thirty days when the new hair had grown in. Unplucked controls of the same genotypes were also exposed to these environmental temperatures and their coat color measured before and after the thirty day exposure period.

2. The subcutaneous temperature of the rump of plucked and unplucked guinea pigs was found to be about 33.3°C and 38.2°C at environmental temperatures of 16°C and 32°C respectively. The rectal temperature of pregnant guinea pigs is about 39.1°C.

3. The growth rate of guinea pig hair at 16°C and 32°C was determined to be about 0.5 mm per day.

4. In blacks (*E-B-C-P-*) there was a doubtfully significant darkening at the low temperature. In albinos of genotype *E-c^ac^aP-* there was highly significant darkening at the low temperature. No other albinos darkened appreciably under either condition. Intermediate combinations of the *C*-locus (c^kc^r, c^dc^r, c^dc^a, c^rc^r, c^rc^a) all darkened and in most cases significantly at both temperatures but more in all cases at the low

temperature and significantly so in c^rc^r and c^dc^a. The usual darkening with age thus does not require a skin temperature below 38°C (only 1°C under that before birth) but the process is either markedly increased or supplemented by an effect of low temperature.

5. The pale sepia genotype *E-B-C-ppF-* lightens with age and low temperature has no effect on this process. *E-B-*c^rc^r*pp* however darkens with exposure to 16°C as compared to 32°C. This effect is probably similar to the darkening of the dark sepia genotypes.

6. The dark brown genotype *E-bbC-Pp* behaves differently from any other genotype tested, darkening more at 32°C than at 16°C. More study on this genotype is needed.

7. Pale brown guinea pigs (*E-bbC-ppF-*) lighten with age but low temperature retards this process indicating a partial balance between lightening with age and darkening due to cold.

8. Anomalous creams (*E-C-ppff*) which lighten with age show a balancing effect similar to pale browns during the experimental period.

9. Fading yellows (*eeC-ff*) lighten markedly with age. There may be a slight partial balancing effect of darkening with cold and lightening during the experimental period.

10. Stable yellow (*eeC-F-*) genotypes lighten with age and show no effect of cold. *eec*^{k}*-F-* genotypes tend to increase the amount of phaeomelanin very slightly with age, independently of cold. The amount of yellow pigment in the hair of *eec*^{d}*-F-* genotypes increases strikingly with a low skin temperature.

11. The formation of eumelanin (sootiness) in normally yellow guinea pigs requires low skin temperature.

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DIFFERENTIAL RESPONSE OF MEIOTIC STAGES IN HABROBRACON EGGS TO NITROGEN MUSTARD[1, 2]

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IN some respects, the mutagenic action of the mustards on Drosophila resembles that of X-rays. Both are nonspecific in their effects (AUERBACH and ROBSON 1946), each induces an approximate five-to-one ratio of recessive lethals to visible deletions (SLYZINSKA and SLYZINSKI 1947), the same stage of spermatogenesis appears to be sensitive to both mutagens (AUERBACH 1953) and breaks are induced at random by them on the chromosome limbs (KAUFMANN *et al.* 1949). Differences in action are also apparent—e.g., X-rays induce a higher proportion of gross rearrangements (AUERBACH 1950), genetic mosaics appear more frequently after mustard exposure (AUERBACH 1950), and KAUFMANN *et al.* (1949) showed that the number of translocations increases in sperm chromosomes a week or two after exposure to mustard (X-irradiation data show no such increase).

It has been conclusively demonstrated in Habrobracon (WHITING 1945a and b), in Sciara (BOZEMAN and METZ 1949), and in Trillium (SPARROW 1948) that contracted meiotic chromosomes in diakinesis, metaphase, and anaphase, are much more sensitive to X-rays than are the more dispersed and relatively immobile prophase chromosomes. In preliminary reports WHITING and VON BORSTEL (1952) and VON BORSTEL (1952) described the action of nitrogen mustard on the oocytes of the parasitic wasp *Habrobracon juglandis* Ashmead. This study is a continuation of that research and is concerned with induced egg lethality and chromosome breakage. The results demonstrate that nitrogen mustard acts like X-rays in that oocytes in the first meiotic metaphase are much more sensitive to these mutagens than are oocytes in the first meiotic prophase. Furthermore, the study indicates that nitrogen mustard differs from X-rays since single chromatid breaks predominate among the aberrations induced by the former whereas isochromatid breaks predominate among the latter (WHITING 1945b).

MATERIALS AND METHODS

Wild-type Habrobracon females of stock #33 were used. The females were bred unmated. Eggs of such females develop parthenogenetically. Adult progeny are haploid males. Since no masking of a recessive lethal factor can occur in haploid organisms, lethals, whether dominant or recessive, are always expressed. This study is concerned with embryo lethals only.

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The method for identifying eggs treated in different stages of the meiotic cycle has been described in detail by WHITING (1945a). Virgin females are well fed on fresh caterpillars (Ephestia larvae) for several days and then kept overnight at 29–30°C without food. Under these conditions oocytes in the late metaphase of the first meiotic division (metaphase I) are stored in the uterine sacs. On the following morning the wasps are exposed to the chemical mutagen and, following treatment, each is set with a previously stung caterpillar in a two-inch Stender dish. After six hours the wasps are removed from the caterpillars, since it is known that eggs laid during this time were in metaphase I when treated. The eggs are counted, and forty hours later hatchability percentage is determined. The females are again kept overnight and then set with stung caterpillars for two hours, after which time they are moved to new dishes containing stung caterpillars and left for five to forty-eight hours more. The eggs collected in the two-hour period include some which were in late prophase I and some which were in metaphase I when exposed to the nitrogen mustard. After irradiation the eggs oviposited during the five-hour period on the day after exposure represent those eggs which were in late prophase I when treated. This schedule must be modified in experiments with nitrogen mustard since females tend to lay their eggs more slowly after exposure to this mutagen. In a few experiments, females which had no metaphase I oocytes were required. These females, with oldest oocytes in prophase I, were obtained by removing them from cocoons just before normal eclosion.

For cytological investigations, the whole-egg technique (WHITING modification of Schmuck-Metz method; 1950) in which the Feulgen procedure was used was employed.

The nitrogen mustard, methyl *bis* (β-chloroethyl)amine hydrochloride (kindly supplied by Merck and Company), was the chemical mutagen used in this investigation. The chemical was prepared as a 10% solution in distilled water and was delivered to the wasps as an aerosol. The period of exposure rather than concentration of the nitrogen mustard was the variable factor in the treatment. The aerosol-generating apparatus used in this study was designed by DR. JEAN KERSCHNER. It is a combination of techniques used by other investigators—the type of nebulizer used by DEMEREC (1948), the mutagen administered at intervals with fresh air as

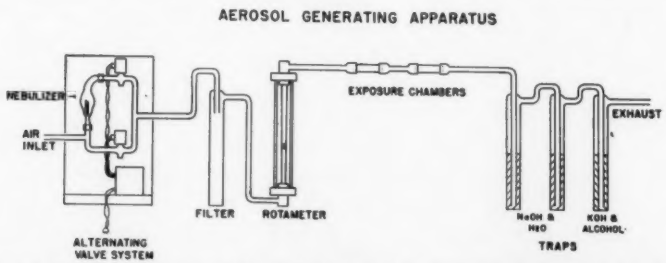

FIGURE 1. Schematic diagram of the aerosol-generating apparatus. The mutagenic solution is placed in the nebulizer and is transmitted as an aerosol to the wasps contained in the exposure chambers.

utilized by AUERBACH and ROBSON (1947a and b), and the traps and filters as suggested by CARR (1947). Since experiments could be reliably repeated, it is worth describing the method in detail. The plan of the apparatus used is shown in figure 1; the direction of flow is as follows: Air compressor—alternate air and aerosol flow—filter (condenser of DEMEREC)—rotameter—exposure chamber or chambers—traps of NaOH in water—trap of KOH in 95% alcohol. The aerosol (produced by a DeVilbiss-40 nebulizer) was blown over the wasps for fifty seconds, and then air was blown over them for ten seconds of each minute. The flow of air alone served to dilute the atmosphere, to evaporate coalesced droplets of the mutagenic solution which may have adhered to the animals or the exposure chamber, and to allow a short recovery of the organisms from the toxic effects of the chemical before the mutagenic treatment was resumed. The filter takes out of circulation the droplets over 1.5 μ in diameter (DEMEREC 1948). Experiments performed without the filter produced inconsistent results. The rotameter (flow meter) was used to measure the flow of air and aerosol in order to maintain a constant dose during the individual experiments and to enable accurate repetition of doses in additional experiments. During the aerosol generation period of 50 seconds, the air flow was left constant at 5.6 liters per minute. The flow of air alone for ten seconds of each minute was delivered at a rate of 25.2 liters per minute. No more than two chambers were used in series at any one time; wasps in the two chambers received very nearly the same dose. A piece of absorbent paper was placed in each exposure chamber. According to CARR (1947) the paper serves to absorb liquids which are extruded by flies when the mutagen reached them; more consistent results were obtained when the paper was present.

RESULTS

The Habrobracon oocyte is most sensitive to the lethal action of nitrogen mustard when the egg nucleus is in the first meiotic metaphase. Data in table 1 show that a treatment period of 5 minutes under the conditions of the experiment yielded a

TABLE 1

Dose-hatchability for eggs exposed to nitrogen mustard during meiotic metaphase I and prophase I (A) Eggs oviposited during a 5-hour period after metaphase I oviposition; (B) Eggs oviposited during a 5-hour period without metaphase I eggs having been previously laid; (C) Eggs oviposited during a 48-hour period after metaphase I oviposition

Exposure (min.)	Metaphase eggs/ female	Percentage hatchability			
		Metaphase I	Prophase I		
			A 5 hr	B Prophase only	C 48 hr.
Control	13.2*	313/328 = 95.5 ± 1.15	81/84 = 96.4 ± 2.06	68/78 = 87.2 ± 3.78	141/146 = 96.6 ± 1.50
5	7.16	19/86 = 22.1 ± 4.48	76/98 = 77.6 ± 4.21		
15	9.49	0/332 = 0	170/269 = 63.2 ± 2.94		
30	10.03	0/587 = 0	186/321 = 57.9 ± 1.76	29/34 = 85.3 ± 6.07	
60	5.15	0/278 = 0	3/63 = 4.76 ± 2.69	46/97 = 47.4 ± 5.07	384/817 = 47.0 ± 1.75
90	6.10	0/238 = 0	2/54 = 3.70 ± 2.57		163/516 = 31.6 ± 2.05

* A. R. Whiting, 1945a.

hatchability percentage of 22.1 for metaphase I oocytes. After a 15-minute exposure none of the eggs hatched. The approximate 100% lethal dose for metaphase I oocytes is probably about 10 minutes.

Oocytes treated in the first meiotic prophase (prophase I) were more resistant to the action of nitrogen mustard (table 1). Total lethality of the prophase I eggs may never be obtained since the females die before they are sterilized. Most of the short exposure experiments were done following the procedure of WHITING (1945a) in which the eggs treated in prophase I were collected over a 5-hour period. It was observed that prophase I hatchabilities (table 1, col. B) were considerably higher when newly eclosed females were treated. Newly eclosed females have no more than one metaphase I oocyte. It was concluded that the lower hatchabilities recorded for the 5-hour collections of prophase I eggs (col. A) were due to retention of metaphase I oocytes. The female wasps were apparently weakened by the chemical mutagen and oviposited less freely than the controls. A calculation of the average number of metaphase I eggs per treated female when compared with control number (table 1) corroborates this point of view. The metaphase I hatchability data are in no way invalidated. For higher doses, the prophase I eggs were collected over a 48-hour period. Only the females which had oviposited fewer than 50 eggs, the approximate number of postsynaptic prophase I oocytes, were included in the tabulation.

Experiments on meiotic stage sensitivity

All experiments were designed so that the eggs which were treated in metaphase I were laid on the day of treatment. The possibility exists that nitrogen mustard may act for only a short time on the oocyte, after which "poisoning" would gradually diminish with time. (The half life of nitrogen mustard in a neutral aqueous solution is approximately 30 minutes, KÖLMARK and WESTERGAARD 1949. Although the 10% solution used on Habrobracon was not neutral, the nitrogen mustard was apparently rapidly dissipated, since delayed mating of treated females demonstrated a lack of mutagenic effect on the sperm, WHITING and VON BORSTEL 1952.) Presumably this would permit eggs laid on the day following treatment (the oocytes treated in prophase I) to recover and thus allow a higher percentage to hatch. The following experiment excluded the possibility that a "temporary effect" occurred, allowing eggs

TABLE 2

Tests for recovery of oocytes. Hatchability of eggs laid on different days after nitrogen mustard treatment using a 30-minute dose

Experiment	Day eggs laid		Percentage hatchability	
	Metaphase I	Prophase I	Metaphase I	Prophase I
IA	1	2	0/170 = 0	42/75 = 56.0 ± 5.73
IB	2	3	0/57 = 0	9/17 = 52.9 ± 12.1
IIA	1	2	0/120 = 0	54/101 = 53.5 ± 4.96
IIB	2	3	0/106 = 0	28/47 = 59.6 ± 7.15
Average			0/453 = 0	133/240 = 55.4 ± 3.12

laid on the second day after treatment to "heal." Table 2 summarizes the data from two experiments in each of which the females of one group (col. A) were allowed to lay their eggs treated in metaphase I on the day of treatment and their eggs treated in prophase I on the day after treatment. The females in the second group (col. B), in each experiment, were put on the caterpillars on the day after treatment to lay their metaphase I eggs; their prophase I eggs were laid two days after treatment. The results of the experiments were consistent throughout. Not one of the eggs treated in metaphase I hatched; 55.4% of the eggs treated in prophase I hatched. Under the conditions of the experiment, there was no evidence for recovery of oocytes after the nitrogen mustard had long been broken down.

In wasps, the mature metaphase I oocytes in the uterine sacs are nearer to the vaginal opening and are oviposited first; the oocytes in prophase I are still growing in the ovarioles. Although the mutagen probably reaches the ovarioles through the tracheoles (DEMEREC 1945), there exists the possibility that the difference in sensitivity to nitrogen mustard treatment between oocytes in metaphase I and prophase I was due to the more ready access of the chemical to the metaphase I oocytes. Some evidence was provided by the following experiment which suggests that differential accessibility is not a factor in determining sensitivity. Hatchability of prophase I eggs from treated females that had no metaphase I eggs was compared with hatchability of eggs from treated females that had stored metaphase I eggs. Table 1 shows that hatchability was not lower for the eggs treated in prophase only (col. B) than for prophase I eggs in the treated metaphase I and prophase I (col. A or C) oocyte group. Therefore, it seems likely that nitrogen mustard does reach the younger oocytes when the oldest are present.

Better evidence was provided by some experiments carried out on Sciara (VON BORSTEL 1953). When a Sciara female ecloses, her eggs are in the early prophase of the primary oocyte stage. During the next 60 hours at 19°C, the eggs proceed synchronously through prophase and metaphase to the early anaphase of the first meiotic division at which stage they remain until laid (REYNOLDS 1941; BOZEMAN and METZ 1949; CROUSE 1950). In Sciara there is no question of differential accessibility since the oocytes are all in the same stage of maturation. Females treated with nitrogen mustard at different stages of the meiotic cycle provided evidence that the oocytes in late metaphase or early anaphase are more sensitive to nitrogen mustard than are the early prophase I oocytes.

Chromosome breakage

SPEICHER (1936) described the stages of oogenesis and cleavage in the unfertilized egg of Habrobracon, and his observations are summarized as follows: The maturation of the unlaid oocyte proceeds to and is interrupted at the first meiotic metaphase, in which stage the oocyte remains until after oviposition when maturation is resumed. During oviposition the first meiotic spindle passes from the dorsal to the ventral side of the egg. The first meiotic division occurs and the chromosomes pass quickly into telophase I. The second meiotic division follows immediately; the four haploid groups of chromosomes (*1a*, *1b*, *2a*, and *2b*) lie consecutively more or less in a line perpendicular to the egg surface. Polar body *1a* quickly degenerates

and polar bodies *1b* and *2a* slowly fuse, while the spherical pronucleus *2b* migrates into the interior of the egg. The nuclear divisions are synchronous and syncitial until occurrence of blastoderm formation at the tenth or eleventh cleavage.

The breakage-fusion-bridge cycle (McCLINTOCK 1938) operates in the meiotic chromosomes of irradiated Habrobracon oocytes (WHITING 1945a and b) and isochromatid breaks can be distinguished from single chromatid breaks. A double chromatid break induced at metaphase I results in a fragment which is often visible in the first meiotic division. A bridge, formed by fusion of broken dyads, can be detected in the second meiotic division; if the bridge is between polar body *2a* and pronucleus *2b*, it appears in all succeeding cleavage divisions. The result of a single chromatid break is not manifest in the first meiotic division, but in the second meiotic division a fragment is left between the separating anaphase chromosomes. Each succeeding cleavage is marked with a bridge if the injured chromosome gets into the pronucleus.

The cytological studies planned for detection of isochromatid fragments in the first meiotic division were made on the anaphase I and metaphase II stages. When fragments occur, they lie between the separated dyad groups. A low percentage (11.9) of the first meiotic divisions possessed discernible fragments, and these were scored as isochromatid breaks (table 3). Fragments and bridges that were detected in anaphase II, during pronucleus formation and migration, or in cleavage I metaphase are recorded under the caption "Meiotic Division II" in table 3. Of the eggs analyzed, chromosome bridges and fragments were found in 32.9%. Either separately or in combination, pronuclear migration and polar body fusion occasionally obscured the constitution of the break (whether single or iso-). Half the definable breaks were single chromatid in nature; however, there probably existed additional single chromatid breaks which were hidden in many of the eggs examined. The round pronucleus often lay near the polar bodies, possibly eclipsing the fragments, or the pronucleus had migrated so far into the interior of the egg that the tiny, single chromatid fragments were obliterated during this process. Teardrop-shaped pronuclei, signifying bridges, were scored as isochromatid breaks (WHITING 1945b and 1948). Nitrogen mustard drastically impeded the chromosome cycle at the beginning of metaphase of cleavage I. With the technique used, very few embryos were obtained which were in early cleavage. Anaphase and telophase of the first cleavage and prophase of the second cleavage were analyzed for bridges and fragments which occurred in cleavage I, and totals are recorded in table 3. Of the 17 eggs examined in cleavage I, 88.2% had definable bridges and fragments. The data are preliminary but significant.

TABLE 3

Cytological observations of meiotic stages following treatment with lethal doses at metaphase I

Stage analyzed	Total analyzed	Iso-chromatid breaks	Single chromatid breaks	Undetermined breaks	Total breaks	Percentage breaks
Meiotic Div. I	42	5	0	0	5	11.9
Meiotic Div. II	79	9	9	8	26	32.9
Cleavage I	17	0	0	15	15	88.2

DISCUSSION

It is useful here to consider the comparative actions of nitrogen mustard and X-rays on the oocytes of Habrobracon. Figure 2 shows dose-hatchability curves for nitrogen mustard and X-rays. The metaphase hatchability curves were superimposed to provide a scale for comparison of the prophase hatchabilities. It is apparent that metaphase I and prophase I show the same relative sensitivity to both nitrogen mustard and X-rays. The hatchability of prophase I oocytes, which were assumed to have had some metaphase I oocytes mixed with them during collection, shows a type of curve which is consistent with the conclusion that metaphase I oocyte retention had occurred.

With metaphase sensitivities used as criteria for comparing effects of X-rays and nitrogen mustard it was observed that sperm were far more sensitive to nitrogen mustard than they were to X-rays (WHITING and VON BORSTEL 1954). It seems likely that sperm nuclei are more readily accessible to nitrogen mustard than are egg nuclei. Consequently, the X-ray data probably give a truer picture of stage sensitivity between nuclei of different cell types. Virtually every morphological and chemical feature which distinguishes metaphase, prophase, and interphase nuclei has been used by investigators in this field as a reason for differences in sensitivity of meiotic and mitotic stages to X-rays (SPARROW 1951). The experiments with nitrogen mustard do not provide an answer but do indicate that any model devised must provide an explanation for the mutagenic action of mustard as well as that of X-rays. Moreover, the phenomenon of differential sensitivity may perhaps be of a general nature; i.e., the relative sensitivity of a given type of meiotic cell to mutagenic agents may be independent of the mutagen but dependent on the stage of the chromosome cycle.

At lethal doses of X-rays, the breakage-fusion-bridge cycle operates in nearly all the exposed eggs (WHITING 1945b). The same seems to be true for lethal doses of nitrogen mustard. However, a striking difference between X-rays and nitrogen mustard resides in their relative abilities to break chromosomes. Figure 3 shows the

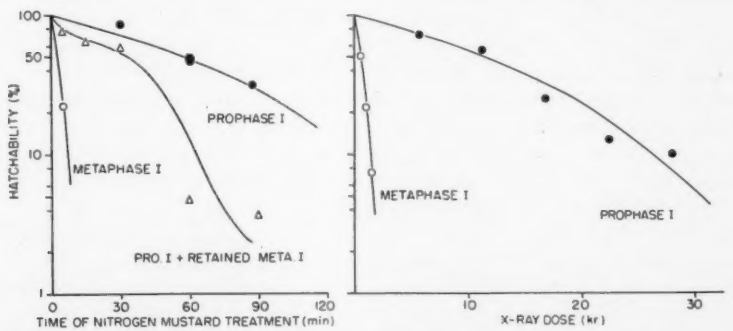

FIGURE 2. Dose-hatchability curves from X-ray and nitrogen mustard experiments comparing the sensitivity of oocytes treated in metaphase I with those treated in prophase I.

comparative effectiveness of lethal doses of nitrogen mustard and X-rays in inducing chromosome breaks at metaphase I in Habrobracon eggs. It should be noted that the degree of injury appearing at the first and second meiotic divisions induced by nitrogen mustard are fewer than with X-rays. However, at cleavage I it becomes clear that the percentages of breaks induced at lethal doses by the respective mutagenic agents are nearly the same.

Conceivably, a preponderance of single chromatid breaks induced by nitrogen mustard (most of the fragments being obscured in the second meiotic division) explains the increase in observed bridges and fragments from 11.9% (metaphase I) to 88.2% (cleavage I). A comparison of eggs treated with lethal doses of nitrogen mustard and X-rays reveals the low frequency of observable fragmentation from

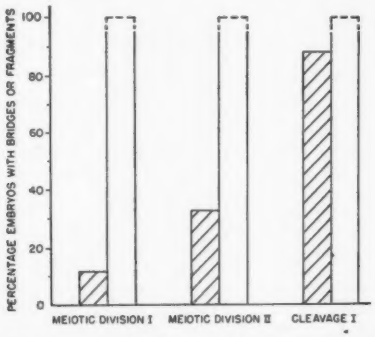

FIGURE 3. A comparison of the mutagenic effects of lethal doses of X-rays with nitrogen mustard; chromosome modifications induced in Habrobracon oocytes treated in metaphase I which appeared at subsequent nuclear divisions.

▨ = induced by nitrogen mustard; □ = induced by X-rays

mustard. The fragments are much smaller than those induced by X-rays, and there is rarely more than one fragment or bridge per egg. Irradiated eggs, on the other hand, often contain fragments which are almost as large as chromosomes, and frequently there are several fragments and bridges per egg. Occasionally, after mustard treatment, bridges are present in the second meiotic division and yet it is impossible to discern any fragments between chromosome groups *1b* and *2a*. It is possible that isochromatid breaks were induced near the ends of chromosomes in these cases; since bridges were never detected in the first meiotic division, all breaks apparently occurred proximal to the terminalized chiasmata.

AUERBACH and ROBSON (1947a) and KAUFMANN *et al.* (1949) observed that sublethal doses of mustard do not produce many gross rearrangements in chromosomes when compared to the number of recessive lethals or small deletions. Drosophila sperm were treated in these experiments and the aberrations were located genetically before observations were made on the salivary gland chromosomes. AUERBACH (1950) suggested that a relative shortage of large rearrangements compared with lethals (SLIZYNSKA and SLIZYNSKI 1947) may be due either to a relative inefficiency of the

treatment to break the chromosomes, or to some secondary effect which interferes with new reunions. She prefers the latter interpretation, but evidence from Habrobracon shows that nitrogen mustard is relatively ineffective in producing isochromatid breaks, and these data would seem to favor the former hypothesis.

As suggested by WHITING (1945b), single ionizations probably cause isochromatid breaks, and such breaks occur frequently. There are some indications that more isochromatid breaks are induced by high doses of nitrogen mustard than by low doses, although dominant lethality is 100% in both cases. This would suggest that a considerable number of inversions and translocations may possibly be induced by nitrogen mustard, but only at doses well above the 100% lethality level. This phenomenon is being more fully investigated.

SUMMARY

(1) An analysis has been made of the induction of total egg lethals by nitrogen mustard in Habrobracon oocytes treated at two different stages of meiosis.

(2) The aerosol-generating apparatus designed for this study was reliable for testing the mutagenic action of nitrogen mustard; results were repeatable, and dose-hatchability data were consistent.

(3) The first meiotic metaphase is approximately 25 times as sensitive to nitrogen mustard as the first meiotic prophase. These data exactly parallel the results found by using X-rays on the same material (WHITING 1945a).

(4) It is suggested that the response to mutagenic agents of meiotic nuclei in a similar cytoplasmic environment may be independent of the mutagen but dependent on the stage of the chromosome cycle.

(5) Chromosome analysis indicates that approximately 100% lethal doses of nitrogen mustard predominantly induce single chromatid breaks. WHITING (1945b) demonstrated that under the same conditions, X-rays predominantly induce isochromatid breaks.

(6) The low ratio of gross rearrangements to recessive lethals found in Drosophila (AUERBACH 1950) may be related to the relative inefficiency of nitrogen mustard in inducing isochromatid breaks.

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GENETIC CONTROL OF HETEROCARYOSIS IN NEUROSPORA CRASSA[1]

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BEADLE and COONRADT (1944) showed that when two different biochemical mutants of *Neurospora crassa*, of the same mating type, were combined on a minimal medium unable to support the growth of either mutant, a heterocaryon with wild type growth rate was formed. The ability to form a heterocaryon between two mutant strains has been taken to indicate that the mutant genes involved are not allelic, although the failure to form a heterocaryon does not necessarily mean that the genes are allelic. The inability to form a heterocaryon may also be due to the impossibility of obtaining a suitable nuclear ratio through the dominance relationships of the genes concerned, or to the action of other genes as reported by GARNJOBST (1953) for inositolless and riboflavinless strains or as described below in a genetically more complex situation involving strains requiring pantothenic acid or lysine.

METHODS

The basal medium was the standard Neurospora minimal (BEADLE and TATUM 1945). All cultures were grown at 25°C. Special growth tubes as described by RYAN, BEADLE and TATUM (1945) were used for growing heterocaryons. Spore suspensions of the strains to be tested for heterocaryon formation were made in sterile distilled water from cultures grown on Horowitz complete medium (HOROWITZ 1947) for 6 days. Loopfuls of each of the two suspensions were then placed together on the surface of the agar in the growth tube. The general procedures for dissection of asci, determining the mating type of cultures, maintaining stock cultures, sterilization of media, etc., were the same as those used by BEADLE and TATUM (1945). Crosses were made on the medium described by WESTERGAARD and MITCHELL (1947), with the addition of appropriate supplements for the mutants concerned.

The mutants used were:—5531 (pantothenicless) TATUM and BEADLE (1942), unable to synthesize pantothenic acid; 1633 (aminobenzoicless) ZIMMER (1946), requiring p-amino-benzoic acid; and 4545 (lysineless)[4] DOERMANN (1946), requiring the amino acid lysine.

EXPERIMENTAL

When the experiments of BEADLE and COONRADT were repeated with reisolates of the same mutants, these being obtained from crosses of the original mutant strains

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to various wild types, heterocaryons were not formed as expected. This suggested that the factors controlling the formation of heterocaryons were more complex than previously thought. After preliminary experiments, the mutants 5531 (pantothenicless) and 4545 (lysineless) were selected to further investigate this problem. Only heterocaryons between strains of the same mating type have been investigated in this work.

5531A was crossed to the EMERSON wild type, E5297a, and 132 pantothenicless random spore isolates from this cross were tested for their ability to form heterocaryons with the lysineless tester strains 4545A and 4545a. Of these cultures 110 failed to form a heterocaryon with the tester strains. Of the 22 strains forming heterocaryons only 11 gave a heterocaryon with wild type growth rate. Following other crosses and after more isolates were tested it was possible to recognize six different types of heterocaryotic growth within this system. These various types were quite constant and classification of any particular growth was readily made into one or the other of the classes. The types may be described as follows (see fig. 1).

Type I

Growth begins within a few hours of inoculation and soon reaches the wild type growth rate (4.4 mm./hr.). The mycelium has the same appearance as wild type and the mycelial frontier is sharp and well defined.

Type II

The mycelial and growth characteristics of this type of heterocaryon are identical with those of type I. However growth does not begin until after a delay of 35–90 hours following inoculation. The commencement of growth is well defined and not gradual.

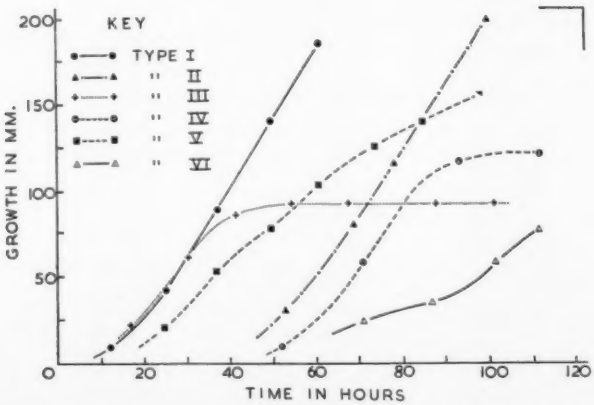

FIGURE 1. The types of heterocaryotic growth observed between different reisolates of 5531 and the tester 4545 strains.

Type III

Growth commences soon after inoculation and with the wild type growth rate, but comes to an abrupt halt after a period of growth varying from 30–60 hours. At the point where growth ceases the mycelium assumes a characteristic highly branched appearance and the immediate edge of the mycelium has a different appearance to the main body of the hyphae, being thinner and closely appressed to the medium. The actual time at which growth ceases is variable even when replicates of the same strains are inoculated. Hyphal tips taken from the region where growth has ceased grow on pantothenic acid supplemented media, but not on media containing lysine. In addition, if pantothenic acid is added to the region where growth has ceased the mycelium is stimulated to grow but no growth occurs if lysine is added in this way. If left for eight to ten days this type of heterocaryon may recommence growth and later stop again. Hyphal tips taken from the actively growing phase of such heterocaryons continue to grow for 24–48 hours after transfer but then stop. Thus this type of heterocaryon appears to be in an unstable state with the pantothenicless nuclei being in excess of the lysineless nuclei.

Type IV

This has the same general growth characteristics as type III with the exception that growth does not begin until 35–90 hours after inoculation.

Type V

Growth begins 12–24 hours after inoculation. The mycelium is much less vigorous than the wild type, is thinner and somewhat appressed to the medium. The mycelial frontier is not well defined. Sometimes the growth becomes progressively thinner, slows down and may eventually cease altogether. The maximum growth rate attained never exceeds 3 mm./hr. and growth is often not linear.

Type VI

The growth characteristics of this type are the same as for type V but like types II and IV growth does not begin until 35–90 hours after inoculation.

Type VII

For uniformity in the scheme, this type includes those strains which do not give any heterocaryotic growth with the tester strains.

Proof of the heterocaryotic condition

The method of BEADLE and COONRADT (1944) was used to prove the heterocaryotic condition and this was readily established for heterocaryon types I, II, V and VI. However it was not possible to use this method for types III and IV. Hyphal tips from these two types show only limited growth, insufficient to enable the heterocaryon to be crossed to wild type. However the hyphal tips from these two types show more growth on minimal medium than that obtained when hyphal tips from either mutant

TABLE 1

Results of intercrossing various heterocaryon types

Heterocaryon types crossed	Total no. asci tested	Results
I x I	15	Progeny all type I.
I x II	18	Progeny consisting of types I and II segregating 1:1 in the ascus.
I x III	16	Progeny consisting of types I and III segregating 1:1 in the ascus.
I x IV	17	Both parental types I and IV and recombinant types II and III recovered in progeny.
I x V	37	Types I and V only recovered, segregating 1:1 in the ascus.
I x VII	23	Types I, II, III, IV, V, VI, VII recovered.
VII x VII	18	All progeny type VII.
III x IV	11	Types III and IV segregating 1:1 in the ascus.

are transferred to minimal medium and this is taken as evidence for the presence of both mutant nuclei in these types of heterocaryon.

From the growth characteristics of the various heterocaryon types a number of features can be recognized which are associated with the formation and growth of a heterocaryon. (1) The establishment of a heterocaryon, (2) the time lag in the initiation of growth, (3) the ability to maintain growth, (4) the growth rate and habit. It has been possible to show that these various characteristics are under genetic control. When the various heterocaryon types are crossed to one another segregation for these types occurs in the progeny. The results of the various crosses are shown in table 1.

On the basis of the data summarised in table 1 and on other evidence to be presented below, the following hypothesis for the genetic control of heterocaryons in *N. crassa* has been formulated. The formation of a heterocaryon between strains of the pantothenicless mutant 5531 and the lysineless mutant 4545 is under the control of genetic factors. These genes control not only whether or not a heterocaryon will form, but the growth characteristics of the heterocaryon, should it form. Four genes have been identified as controlling these characteristics. This represents the minimum required to fit most of the present data, and it is likely that additional genes affect the process. These four genes can be described in terms of their effects as follows:—

Gene W (*alternative allele W'*). Heterocaryotic growth can only occur in the presence of the *W* allele of this gene. However allele *W* alone is not sufficient and an interaction between this allele and alleles *X* and *Y* of the gene pairs *X*/*X'*, *Y*/*Y'* (described below) occurs, so that allele *W* must be present with either of alleles *X* or *Y* for a heterocaryon to form.

Gene X (*alternative allele X'*). Allele *X* of this gene exerts an influence on the growth rate and general vigour of the heterocaryotic mycelium. Segregation of this gene and its alternative allele *X'* has been demonstrated. Pantothenicless strains giving

type V heterocaryotic growth with the tester strains differ from those giving type I by the presence of allele *X'* in the former strains and in crosses of these two types a 1:1 segregation of the parental types occurs in the ascal progeny.

Gene Y (*alternative allele Y'*). Allele *Y* of this gene is concerned with the maintenance of the heterocaryotic condition. Pantothenicless strains giving type III heterocaryons with the tester strains differ from those giving type I growth by having the alternative allele *Y'* and crosses between these two types result in only the parental types occurring in the progeny, segregating 1:1 in the ascus.

Gene Z (*alternative allele Z'*). The presence of the *Z* allele of this gene enables any heterocaryon formed to commence growing soon after inoculation but in the presence of the alternative allele *Z'* growth will be delayed, as in types II, IV, and VI.

It is not known which of the above alleles of these various genes is the dominant form. The only test for dominance in Neurospora is the heterocaryon test so that the dominance or recessiveness of genes affecting the process of heterocaryon formation cannot be determined.

On the basis of this scheme genotypes, with respect to the four genes, of pantothenicless strains giving the various heterocaryon types can be formulated as follows:

I	*W X Y Z*
II	*W X Y Z'*
III	*W X Y'Z*
IV	*W X Y'Z'*
V	*W X'Y Z*
VI	*W X'Y Z'*
VII	*W'* – – –
	– *X'Y'*–

As previously mentioned the genetic constitution of the tester strain will undoubtedly be of importance. To investigate this would require an analysis of the lysineless strains in much the same way as that made for the pantothenicless strains. This is probably not possible because crosses in which both parents carry the lysineless gene are highly infertile, and so the analysis would have to be made through crosses to wild types and this would be both difficult and tedious. Clearly, the genotypes assigned above are valid only for the particular tester strains used.

In order that the segregation and phenotypic effects of the various genes postulated to affect heterocaryon formation may be studied it is necessary that the alternative alleles to the active alleles be introduced into the strains to be analysed. As 5531 evidently carried all the genes necessary to form a type I heterocaryon the first step in the genetic analysis is to cross this strain to another which could be expected to carry the alternative alleles of the various genes. As described above, the wild type EMERSON wild type E5297a was selected for this purpose and crossed to 5531A. The analysis of such a cross is limited by the fact that half the progeny are wild type and thus cannot be tested for heterocaryon formation. If both parents carry the pantothenicless allele then all spore pairs of the ascus may be examined for the ability to form a heterocaryon. If one parent of the cross is of type I, such as 5531, it is obvious then that the segregation of the maximum number of genes affecting heterocaryon can be obtained by selecting a type VII (i.e. a pantothenicless strain unable to form a

heterocaryon with the tester strain) as the other parent. Accordingly a strain of this type, 449-8a, was selected from the progeny of the cross 5531A x 5297a and then crossed to 5531A. The hypothesis described above is based on the subsequent analysis of progeny resulting from this cross.

From the progeny of the cross 449-8a x 5531 all heterocaryon types I through VII were recovered. On the basis of the four gene hypothesis 28 types of asci with respect to the seven different types of heterocaryons are possible. Of 23 asci analysed, 12 of these types, and no others, were obtained. The segregation of the four genes was demonstrated by taking asci showing various segregations of heterocaryon types and making crosses between the various ascospore pairs within each ascus. Two such asci were analysed in this way, asci 560 and 562, both from the cross 449-8a x 5531A.

Analysis of ascus 560

The segregation of the ability to form a heterocaryon in ascus 560 is shown in table 2. All crosses possible between members of the ascus and crosses to the parents were made. The progeny were then tested against the standard 4545 tester strain of like mating type. The results of the various crosses were as follows:—

560-1 x 560-3.—Only segregation for W/W' can be predicted for this cross. Twenty asci were analysed with the results seen in table 3. As only phenotypes III, IV, and VII occur in this cross it is likely that it is heterozygous for W/W' and homozygous for both X and Y'. As types III and IV occur it must be heterozygous for Z/Z'. There is a 1:1 segregation for the ability to form a heterocaryon. On the basis of this cross we can assign the genotype $W'XY'Z$ to 560-3 and so 560-7 must be of genotype $W'X'YZ'$. This can be confirmed by the other crosses.

560-1 x 560-7.—On the basis of the previous cross, this cross should be heterozygous for W/W', X/X', Y/Y' and homozygous for Z'. Thus only types II, IV, VI and VII should appear in the progeny, and this was found to be the case (see table 4). The results from this cross are consistent with the previously postulated genotypes for the spore pairs of ascus 560.

It has previously been postulated that genes W, X and Y interact so that for a heterocaryon to form, gene W and either or both of genes X and Y must be present. The cross supplies evidence for this view. Of the 19 asci analysed from this cross, 10 show segregation for one pair able to form a heterocaryon and three pairs unable to do this, and three asci show segregation of four pairs unable to form a heterocaryon. As the cross is heterozygous for W/W', then two ascospore pairs in each ascus will be of type VII and be unable to form a heterocaryon. When a third or fourth ascospore pair also fail to form a heterocaryon the genotype of these members must contain the W allele and hence failure to form a heterocaryon must be due either to a fifth gene controlling the ability to form heterocaryons or to an interaction between alleles W, X and Y. As no evidence has been found to indicate a fifth gene with this postulated effect and the results can be completely accounted for by assuming the interaction, this has been the explanation adopted. This cross also supplies evidence that the allelic pair Z/Z' does not effect the actual formation of a heterocaryon but only the time at which heterocaryotic growth begins. The formation of heterocaryons or the failure to do so can be completely explained in terms of genes W, X and Y.

TABLES 2 TO 11

Genetic analyses of two asci from the cross 449-8a (pantothenicless, type VII) x 5531-A (panthothenicless, type I)

Numbers of ascospore pairs producing different types of heterocaryons when combined with standard lysineless strains (4545A and 4545a) of like mating type

Cross	Number asci	Type I	Type II	Type III	Type IV	Type V	Type VI	Type VII
		Postulated genotype						
		$\frac{W\ X}{Y\ Z}$	$\frac{W\ X}{Y\ Z'}$	$\frac{W\ X}{Y'\ Z}$	$\frac{W\ X}{Y'\ Z'}$	$\frac{W\ X'}{Y\ Z}$	$\frac{W\ X'}{Y\ Z'}$	$\frac{W'\text{—}^{**}}{-X'Y'-}$
449-8a x 5531-A Table 2	1				1 (560-1a)	1 (560-5a)		2 (560-3A, 560-7A)
560-1a x 560-3A Table 3	2 13 5			2 1	 1 2			2 2 2
560-1a x 560-7A Table 4	4 1 3 3 3 2 3		1 1 1		1 1 1		 1 1 1 	2 2 2 3 3 3 4
560-5a x 560-7A Table 5	4 3 12					2 1	 2 1	2 2 2
560 3A x 560-5a Table 6	1 1 2 2 3 5 1	2 1 1 1		 1 1 1 		 1 1 1	 1* 1* 1*	2 2 2 2 2 2 2
560-1a x 5531-A Table 7	2 7 8	2 1	 2 1	 2 1	2 1			
449-8a x 5331-A Table 8	1	1 (562-7a)				1 (562-5A)		2 (562-1a, 562-3A)

TABLES 2–11—*continued*

Cross	Number asci	Type						
		I	II	III	IV	V	VI	VII
		Postulated genotype						
		$\frac{W\ X}{Y\ Z}$	$\frac{W\ X}{Y\ Z'}$	$\frac{W\ X}{Y'\ Z}$	$\frac{W\ X}{Y'\ Z'}$	$\frac{W\ X'}{Y\ Z}$	$\frac{W\ X'}{Y\ Z'}$	$\frac{W'—**}{-X'\ Y'-}$
562-3A x 562-7a	1	1	1					2
Table 9	1	1			1			2
	1	1				1		2
	1			2				2
	2			1	1			2
	1			1		1		2
	1			1			1	2
	2		1					3
	2			1				3
	1				1			3
	1					1		3
	1						1	3
562-1a x 5531-A	2	1	1					2
Table 10	3	1		1				2
	1		2					2
	4		1	1				2
	1			2				2
	3			1	1			2
562-5A x 449-8a	1					2		2
$W'X'Y'Z'$	3					1	1	2
Table 11	2						2	2
	2					1		3
	3						1	3
	1							4

* Type VI unexpected segregant.

** Type VII phenotype can be: $W'XYZ$, $W'XYZ'$, $W'XY'Z$, $W'XY'Z'$, $W'X'YZ$, $W'X'YZ'$, $W'X'Y'Z$, $W'X'Y'Z'$, $WX'Y'Z$, or $WX'Y'Z'$.

560-5 x 560-7.—On the basis of the previously assigned genotypes, W/W' and Z/Z' are heterozygous and alleles X' and Y are homozygous in this cross. Thus only types V, VI and VII should be obtained in the progeny, and a 1:1 segregation of ability to form a heterocaryon should occur in the ascus. This was the result obtained (table 5).

560-3 x 560-5.—This cross is heterozygous for allelic pairs W/W', X/X' and Y/Y' and homozygous for Z. Thus only types I, III, V and VII should appear in the progeny. This expectation was not borne out experimentally and a number of asci

showed segregation for type VI. The various segregations observed are shown in table 6.

The presence of all the expected heterocaryon types in this and other crosses confirms the four gene hypothesis of heterocaryon formation. The occurrence of type VI amongst the progeny can be accounted for by postulating an additional gene, the action of which is similar to that of the allelic pair Z/Z', but acting specifically on type V heterocaryons. The allele of the gene causing delayed growth can only be detected in the presence of strains giving the type V response. The cross is evidently heterozygous for this gene. If the allele causing delayed growth is carried by 560-3 then the cross to 560-1 would not allow its expression because the progeny of this cross does not include any strains giving type V heterocaryotic growth with the tester strains. If this allele is carried by 560-5 then in the cross 560-5 x 560-7 its presence would not be detected as the cross is heterozygous for alleles Z/Z' and type VI heterocaryons are expected in the progeny of this cross. Thus the complete segregation of this postulated gene could not be followed. Effects attributable to this gene were not detected in any other cross, so the presence and effects of this fifth gene must remain presumptive.

560-1 x 553-1.—This cross is heterozygous for both Y/Y' and Z/Z' and homozygous for W and X, and the observed segregation for types I, II, III, and IV only and in the ascal configurations expected is additional confirmation of the four gene hypothesis (see table 7).

560-5 x 5531.—Here the only allelic pair segregating is X/X' and the 17 asci analysed showed a 1:1 segregation for heterocaryon types I and V when combined with the tester strains.

560-3 x 449-8.—This cross is homozygous for W'. As expected therefore all progeny were of type VII, i.e. unable to form a heterocaryon with the tester strain.

560-7 x 449-8.—All progeny of this cross failed to form heterocaryons with the tester strains. From the genotypes of the parents, this cross is homozygous for both W' and X', so this was the expected result.

Analysis of ascus 562

An analysis similar to that made for ascus 560 was carried out with ascus 562. While the analysis was not so detailed it is sufficient to show the segregation of the four genes already postulated. When members of ascus 562 were tested against the tester strains for heterocaryon formation the responses shown in table 8 were obtained. It is seen that genotypes may be assigned on the basis of these results in a much more complete manner than was possible with ascus 560. This distribution can be completed and confirmed by making various crosses with the parents and between the various spore members. The results of these crosses will now be considered in turn.

562-1 x 562-3.—Eleven asci were analysed from this cross. All the progeny failed to form heterocaryons with the tester strains. This result is consistent with the assigned genotypes for 562-1 and 562-3. This cross gives no information about the segregation of alleles X/X', but this is supplied by several of the other crosses.

562-1 x 562-5.—The ascospores from this cross showed extremely poor germina-

tion and although over forty asci were dissected none gave germination in all four spore pairs thus preventing the analysis of this cross.

562-3 x 562-7.—Fifteen asci were analysed from this cross with the results given in table 9. It is seen that all heterocaryon types I through VII occur in the progeny, and hence all four genes must be heterozygous in this cross. As 562-7 is type I and hence of genotype *W X Y Z*, then 562-3 must have the genotype *W'X'Y'Z'*. Here again we have additional evidence for the interaction between genes *W*, *X* and *Y* to give the type of ascus in which more than two spore pairs show the type VII response. From the results of this cross we now assign genotypes to all members of this ascus, 562-1 *W'XY'Z'*, 562-3 *W'X'Y'Z'*, 562-5 *WX'YZ*, 562-7 *WXYZ*. Additional evidence for this segregation comes from the following crosses.

562-1 x 5531.—Fourteen asci were analysed from this cross with the results given in table 10. From the results of the cross 562-3 x 562-7 we have assigned the genotype (*W'XY'Z'*) to 562-1. Hence in the cross of this latter strain to 5531, which has the genotype *WXYZ*, we expect to find only types I, II, III, IV and VII. This was found to be the case and the results of this cross confirm the genotypes assigned on the basis of the previous cross.

562-5 x 449-8.—This cross completed the analysis of ascus 562. The results from this cross are given in table 11. This cross is between genotypes *WX'YZ* and *W'X'Y'Z'* and the expected segregation for types V, VI, and VII only is realized. This cross supplies additional confirmatory evidence for the interaction occurring between *W*, *X*, and *Y* in the formation of a heterocaryon.

No further evidence was found in the analysis of ascus 562 for the additional gene previously postulated to act specifically on the type V heterocaryon to produce the type VI heterocaryon.

The results of the analysis of both asci 560 and 562 thus confirm the experimental hypothesis for the genetic control of heterocaryon formation and growth. Preliminary experiments with other mutants suggest that the results obtained with the lysineless-pantothenicless heterocaryon are of general application in the formation of heterocaryons between biochemical mutants but these tests were not carried far enough to indicate the number of genes involved nor their relationship, if any, to genes *W*, *X*, *Y* and *Z*.

Influence of the tester strain on the type of heterocaryotic growth

It has been shown above that the genotype of one partner in the combination of two strains to form a heterocaryon will determine the nature of the heterocaryotic growth resulting or may even prevent heterocaryon formation. It is thus likely that the genotype of the second strain will also exert a similar influence. Thus four genes carried by the pantothenicless partner have been shown to influence heterocaryon formation so that it is quite reasonable to expect that perhaps a similar number is carried by the lysineless component. It would be also expected that such genes may have a complementary action. Although the exact nature of the genetic control exerted by the lysine strain is not known experiments have been carried out which demonstrate that the genotype of the lysine strain does affect the subsequent heterocaryotic growth.

TABLE 12

Influence of different lysineless tester strains on the formation of heterocaryon types with various pantothenicless strains

Pantothenic strain	Heterocaryon types formed					
	4545 A standard tester	Random spores from cross 4545A x 25a, mating type 'A'				
		L102	L104	L106	L109	L112
741-7A	I	II	II	I	IV	III
726-5A	II	II	III	II	II	II
731-5A	III	II	II	III	II	II
742-4A	IV	V	VI	IV	VII	V

A number of random spores were selected from the progeny of the cross 4545A x 25a (LINDEGREN wild type). They were combined with different pantothenicless strains of like mating type, the latter known to form heterocaryons of various types with the standard tester strains. The results are given in table 12.

These results indicate that the genetic hypothesis advanced for the control of heterocaryosis between these two mutants is true only when the tester strains used in the experiments are involved. Had other tester strains been used it is clear that the hypothesis would have to be modified or extended. It is of interest to note the comparatively large number of delayed starting types of heterocaryons (II, IV and VI) obtained in the above results. This supports the previous suggestion that the inheritance of this character is complex. Another interesting result from the above experiment is that apparently strain L106 carries the same genic complement with respect to the genes under consideration as does 4545A, because both give the same results with the four strains tested.

Apparently the two tester strains 4545a and 4545A are alike with respect to the principal genes controlling heterocaryosis. In all the crosses tested the range of types obtained amongst the progeny were the same for either mating type and no results suggested that 4545A and 4545a were not isogenic for the genes under consideration.

DISCUSSION

From the evidence presented above it may be concluded that certain genes control the formation of heterocaryons between biochemical mutants of *Neurospora crassa*.

Little can be said regarding the mode of action of genes affecting heterocaryosis. Although this aspect of the problem has not been investigated, certain suggestions can be made. The first situation to be considered is that of the formation of heterocaryons between some strains and not between others. Since the first stage in the formation of a heterocaryon is presumably the fusion of two germinating spores it should be possible to determine by appropriate micro-techniques whether failure to form a heterocaryon is due to an initial failure to fuse. In the formation of some heterocaryons it is apparent that fusion can and does take place quite soon after inoculation. With some type I heterocaryons growth is visible within a matter of hours after inoculation and the initiation of growth is just as rapid as with wild type strains.

Experiments were done in which two strains known not to form a heterocaryon were grown alone on agar plates supplemented with sub-optimal requirements of the specific growth requirements. Squares were then cut from the agar containing growing hyphae of each strain and placed in apposition at one end of a growth tube containing minimal medium. Thus the strains had every opportunity to fuse but in no case where this was tried did heterocaryotic growth result. Similar experiments in which the specific substrate was incorporated with the inoculum of spores failed to have any effect in initiating heterocaryotic growth.

In those heterocaryons which show delayed growth it is not known whether this is due to a delay in fusing or a delay following fusion. Inocula of such combinations examined under the microscope seem to show no more germination than that observed with either strain inoculated by itself so the former situation may be the case.

There is no reason to believe that the four (or possibly five) genes shown above to be concerned in the formation of the lysineless-pantothenicless heterocaryon represents the total number of genes capable of exerting such an effect. Furthermore it is not known whether these genes are specific in their action for the particular mutants concerned or whether they can affect other heterocaryotic systems. GARNJOBST (1953) has shown that the genes *het-1* and *het-2* can influence the heterocaryon forming ability of a number of biochemical mutants. Unfortunately it is not possible at present to relate these two genes to any of those described above for the pantothenicless-lysineless system.

SUMMARY

Heterocaryon formation between certain biochemical mutants of *Neurospora crassa* is controlled by a number of genes apart from the biochemical mutant genes concerned. Four genes, and possibly a fifth, have been shown to be concerned in the process of heterocaryon formation between a mutant requiring pantothenic acid and one requiring lysine. These genes may not only prevent the formation of a heterocaryon but may also modify the type of heterocaryotic growth. The growth characteristics so affected are the time at which heterocaryotic growth commences, the ability to maintain heterocaryotic growth, and the vigor of the growth.

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GENETIC MODIFICATION OF A TEMPERATURE SENSITIVE LETHAL IN DROSOPHILA WILLISTONI

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THE SURVIVAL value of a genetic factor is conditioned by its phenotypic manifestation in all environments encountered by the species, as well as by its performance in combination with the rest of the genotype of the species. The hereditary norm of reaction can be analyzed by observing a given factor under various environmental conditions and in different genotypes. DOBZHANSKY and SPASSKY (1944) studied the expression of characters associated with the second and fourth chromosomes of *Drosophila pseudoobscura* by changing the environment as well as the residual genotype. They found one chromosomal type which had an almost normal viability at 16½°C, was semilethal at 21°C and lethal at 25½°C. Various degrees of viability of this chromosome were obtained at 21° by altering the genetic background. Another factor on the fourth chromosome exhibited temperature-conditioned visible characters which could be paralleled by changes in genetic residues. Viability influences of five systems of geographic modifiers were tested for a number of homozygotes for second and third chromosomes derived from natural populations. Some modifiers caused larger viability changes than others, but the influence of a particular geographic modifying system was not beneficial to all homozygotes nor deleterious to all.

A temperature-sensitive lethal factor (*2WB*), the viability effect of which can also be modified by alterations in the genotype, has been discovered in *Drosophila willistoni*. The recessive factor *2WB* on the second chromosome was extracted from the Goyaz (Brazil) population of *D. willistoni*. At 25°C this factor is a lethal. The *2WB* homozygotes are viable below 25°C and can be distinguished by their wing and eye abnormalities. The wings of the newly emerged flies are blistered and vesiculated, but in older flies they appear shrunken and slightly darker in color than normal wings. The wings are held in the unfolded position (divergent). The description of *balloon wings* in *D. melanogaster* fits this lethal more closely than any other described mutant (BRIDGES and BREHME 1944). Irregular curvature of the eyes also occurs in the *2WB* homozygotes. A pure culture of the abnormal flies has been maintained at 18°C.

The balanced lethal stock of *2WB* was outcrossed to a wild strain, "Standard"; the *2WB*/+ flies obtained in the F_1 were mated and their offspring raised at 25°C. Among the progeny of the F_2 there were flies with shrunken wings which had survived the lethal temperature. Repetition of this experiment revealed discrepancies in the percent survival of the shrunken flies in the F_2. These preliminary observations raised several questions. One problem concerns the nature of the factors which are

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responsible for the modification of the *2WB* norm. Are these results due to the presence of genetic modifiers affecting the viability of the *2WB/2WB* zygotes or is the appearance of the shrunken flies at the higher temperatures a result of crossing over between a lethal factor and a factor for shrunken wings? Furthermore, what is the geographic distribution of these viability factors in Brazilian populations of *D. willistoni* with respect to the population which gave rise to *2WB*? Since the genetic variability and biology of numerous Brazilian populations of *D. willistoni* have been so extensively examined (BURLA *et al.* 1949; SPASSKY and DOBZHANSKY 1950; PAVAN *et al.* 1951) it was considered desirable to study the geographic distribution of the modifiers of the *2WB* factor. Such analyses may provide information about the evolutionary significance of the concealed genetic variability found in natural populations.

METHODS

In order to determine whether the shrunken flies are the result of crossing over between the lethal factor and a shrunken factor, or whether the change in the vi-

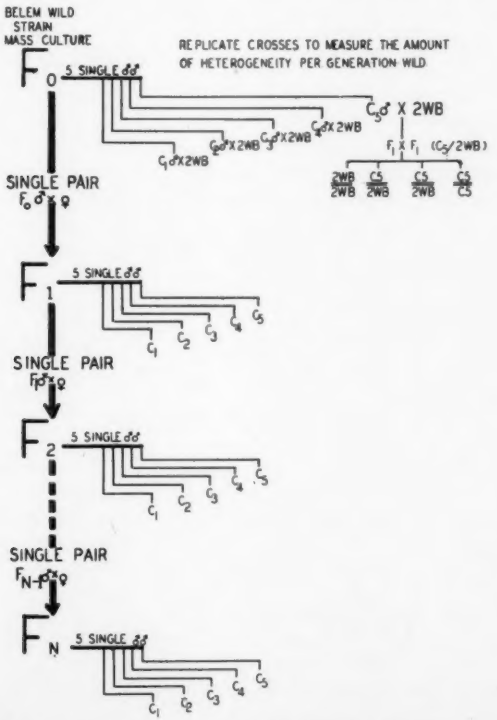

FIGURE 1. Scheme of replicate crosses to measure the amount of heterogeneity in the Belem wild strain and the effect of inbreeding.

ability of the shrunken flies is due to the presence of genetic modifiers in the wild strain, the principle of inbreeding was employed. It was argued that inbreeding a wild strain of flies for a number of generations and testing by replicate crosses for its reaction with the *2WB* norm after each inbred generation would distinguish between the two alternatives. If inbreeding increases the genetic homogeneity within the wild strain, then the variability of replicate crosses of the wild strain to the *2WB* stock should decrease with each subsequent generation of inbreeding. Such results would indicate that genetic modifiers of the wild strain must be responsible for the viability changes of *2WB*. Recombination, however, should show no regular trend of increasing homogeneity.

The factor *2WB* had been maintained in the laboratory in a balanced lethal system for three years before the present experiment was begun. To further insure the homogeneity in the *2WB* stock, it was inbred for eight generations by single pair matings. Then the crosses outlined in figure 1 were made. A single male (F_0, C_1) from the Belem wild strain was crossed to a group of *2WB*/Balancer females. The F_1 *2WB*/+ flies were mated and placed in egg collection chambers. From this cross 600 F_2 larvae were collected and raised in creamers (20 larvae per creamer) on cream of wheat-molasses medium (SPASSKY 1943). The method of egg and larval collection was that previously described by RIZKI (1952) for *D. willistoni*. Five replicates of this series of crosses were made (C_1–C_5), and 600 larvae were obtained from each. The larvae were raised at a constant temperature of 26 ± 0.25°C. Every precaution was taken to equalize the environmental conditions throughout the experiment from creamer to creamer. Mortalities were also taken into account by running normal controls under the same conditions. The pupae in each creamer were counted and the adults were classified according to sex and wing type (normal and shrunken). This scheme was repeated for the subsequent inbred generations of the Belem wild strain; each successive generation of Belem wild strain used was the result of a single pair mating of the preceding generation.

The χ^2 test for heterogeneity was applied to the data. At the outset of the experiment it was decided to discontinue inbreeding the Belem wild strain when the χ^2 value between replicates for a given generation changed from significant to nonsignificant and remained so for the next generation; otherwise the inbreeding should be continued for at least six generations to note any trend.

In addition to the Belem wild strain several males of each of the following wild strains were crossed to *2WB*/Balancer females and the percent shrunken flies in the F_2 from each cross was determined: Iguassu, Palma, Imperatriz, Marajo, Rio Negro, Bolivia and Içana.

RESULTS

The data from the inbreeding experiments of the Belem wild strain are presented in table 1. The χ^2 test reveals considerable heterogeneity among the Belem males (C_1–C_5) of the F_0 and F_1 inbred strain with regard to modification of *2WB*. But the nonsignificant χ^2 value obtained by testing the five males of the F_2 inbred generation indicates homogeneity which is retained in the F_3 generation. The trend toward homogeneity in the modification of *2WB* by inbreeding the wild strain is also apparent in the variance of the ratio of normal to shrunken wing per generation (table 2).

TABLE 1

Measurement of heterogeneity of the Belem strain

		Normal		Shrunken		Total	$\chi^2_{3d.f.}$	P
		♂♂	♀♀	♂♂	♀♀			
F_0	C1	160	156	83	33	432		
	C2	155	151	77	48	431		
	C3	135	156	77	43	411		
	C4	141	142	79	79	441		
	C5	181	173	61	37	452		
	Total	772	778	377	240	2167		
							40.30	<0.0001
F_1	C1	106	168	42	51	367		
	C2	197	184	64	37	482		
	C3	192	142	51	52	437		
	C4	209	177	27	34	447		
	C5	187	175	59	64	485		
	Total	891	846	243	238	2218		
							56.66	<0.0001
F_2	C1	192	194	63	74	523		
	C2	173	177	75	76	501		
	C3	172	162	51	50	435		
	C4	153	173	67	63	456		
	C5	148	175	87	75	485		
	Total	838	881	343	338	2400		
							18.37	≃0.12
F_3	C1	217	178	31	33	459		
	C2	184	155	36	34	409		
	C3	202	164	29	25	420		
	C4	167	181	19	25	392		
	C5	167	160	29	31	387		
	Total	937	838	144	148	2067		
							13.30	≃0.35
Repeat F_1	C1	195	180	24	32	431		
	C2	189	156	50	27	422		
	C3	192	189	15	16	412		
	C4	199	185	15	16	415		
	C5	199	173	19	12	403		
	Total	974	883	123	103	2083		
							50.10	<0.0001

TABLE 2

The Trend of Increasing Homogeneity of 2WB *Modification by Inbreeding the Belem Strain as Shown By the Variance,* S^2

	$\bar{X}_c$	S^2	N
F_0	0.715	0.002531	5
F_1	0.783	0.003759	5
Repeat F_1	0.890	0.002175	5
F_2	0.716	0.001475	5
F_3	0.859	0.000305	5

This homogeneity in the modification of *2WB* obtained by inbreeding implies the presence of genetic modifiers of *2WB* in the Belem strain.

The crosses (C_1–C_5) of the F_1 generation were repeated by raising a new line from the F_0 (mass culture) by single pair mating. The χ^2 value shows heterogeneity among the five males as in the sample of the original F_1. The presence of heterogeneity in the original Belem strain is confirmed. (The difference in ratios of normal to shrunken flies in the original F_1 and the repeat F_1 is not clear on the basis of the present experiment. It may be due to the heterogeneity and the initial number of modifiers in the F_0. Also compare the ratio of normal and mutant individuals in the repeat F_1 with F_3.)

Additional evidence that the shunken flies are carrying the "lethal" *2WB* factor has been obtained from data on zygotic mortalities. Mortality of the Belem wild strain was determined under the same experimental conditions as the mortality occurring in the cross *2WB* x F_0 Belem. The total mortality of the Belem wild strain was 18% as compared with an average mortality of 25% from the cross of the Belem strain to *2WB*. This difference of 7% does not account for the presence of a recessive lethal factor which has an expected mortality of 25%.

Furthermore an average of 0.29 of the progeny from the cross *2WB* x F_0 generation of the Belem wild strain had shrunken wings. Theoretically such a high proportion of flies with shrunken wings would not be expected as a result of crossing over. If the two factors are completely separable and independent from one another then the expected ratio of normal to shrunken flies is 5:1. Any linkage between the factors would decrease the proportion of shrunken flies.

TABLE 3

Percentage of shrunken flies obtained by crossing 2WB *to various wild strains of D. willistoni*

Wild strain	No. larvae	No. adults	Fraction shrunken	
			p	σ
Iguassu	580	400	0.14	± 0.017
Palma	580	352	0.09	± 0.015
Imperatriz	580	292	0.08	± 0.016
Marajo	580	411	0.08	± 0.013
Rio Negro	600	412	0.05	± 0.011
Bolivia	600	416	0.07	± 0.013
Içana	600	381	0.06	± 0.012

In addition to the Belem wild strain seven other wild strains of *D. willistoni* were crossed to the *2W B* stock and the offspring raised at the lethal temperature. Shrunken flies were obtained in the F_2 generation from all of these crosses ranging in proportion from 0.05 to 0.14. The data are summarized in table 3. The cross with the Iguassu wild strain gave 0.14 shrunken flies; this value is significantly different from that of the Belem wild strain which gave the highest proportion of shrunken flies, 0.29. The *t* value for the comparison of Palma and Rio Negro indicates significance. Since the results from the Rio Negro population are not significantly different from those of Marajo and Imperatriz which in turn are not different from Palma, then it may be concluded that the difference between Palma and Rio Negro is not highly significant.

DISCUSSION

Environmental conditions can alter the viability of the *2WB* factor. At 25°C this factor is a lethal, but at lower temperatures viable phenotypes result from the same genotype. Changes in the residual genotype also can produce viability changes of the *2WB* factor, and depending upon the modifying system, the lethal factor *2WB* may become a semilethal or viable. Most of the wild strains tested showed modification to the level of semilethality. It should be stressed that in these experiments on genetic modification every precaution was taken to reduce environmental fluctuations between crosses to a minimum. The inbreeding experiments were designed to determine whether the modification of *2WB* by a wild strain is the result of recombination of genetic factors or whether genetic modifiers of *2WB* exist in the wild strain. The observed results are best explained by the occurrence of genetic modifiers.

The time of death of the lethal homozygotes of *2W B* is very close to the stage of emergence of the imagines from the puparium. There is an upset in the hatching movement and time of emergence. The wings of the lethals begin expanding while the fly is still in the puparium and become filled with haemolymph. This haemolymph in the wings forms blisters which often rupture while the fly is enclosed in the pupal case. The excessive loss of haemolymph and desiccation, or the entanglement of the imago in the puparium, or both factors may be considered the final cause of the lethality. It is conceivable that a slight change in the timing or rate of action of the factors involved in emergence induced either by alterations in the environment or residual genotype can shift the individual zygote into the lethal or the viable class.

Of the eight wild strains of *D. willistoni* which were crossed to the *2WB* stock, Belem and Iguassu gave higher proportions of shrunken flies than all of the others. The highest proportion of shrunken wings, 0.29, resulted from the cross to the Belem strain, 0.14 were found in the Iguassu cross, and values ranging from 0.05–0.09 occurred in the other strains.

The *2WB* factor originated in the *D. willistoni* population of Goyaz (Brazil). No correlation was found between the distance of the wild strains from the point of origin of the lethal factor and the amount of modification of *2WB*. The distribution of the genetic modifiers is geographically random. There is also no correlation between the mean number of inversion heterozygotes per fly in different populations and the degree of modification of the *2W B* norm (DOBZHANSKY *et al.* 1950; CUNHA *et al.* 1950). However, there does seem to be a possible correlation between the fre-

quencies of lethals and semilethals in different populations of *willistoni* and the degree of modification of viability of *2WB* in the present experiment. PAVAN *et al.* (1951) in an extensive study of concealed genetic variability in Brazilian populations of *D. willistoni* found that, "it seems that the populations of central and northern Brazil, except that of Para carry more lethals and semilethals than those in the south" (Para = Belem). Of the southern populations studied by Pavan, Iguassu had a low frequency of lethals and semilethals and gave high modification of *2WB*. Belem is in northern Brazil, but is the exceptional case in Pavan's study by having lower frequencies of lethals and semilethals; in the present experiment Belem gave high modification of *2WB*.

SUMMARY

1. The recessive factor *2WB* is a lethal at 25°C whereas at lower temperatures the *2WB* homozygotes are viable and are recognizable by their shrunken wings. Shrunken flies are also obtained among the progeny of crosses of the *2WB* stock to various wild strains of *D. willistoni* raised at 25°C.

2. It has been demonstrated that the survival of the "lethal" homozygotes of *2WB* in combination with the Belem wild genotype is due to the action of viability modifiers present in the Belem strain.

3. Eight wild strains of *D. willistoni* were crossed to the *2WB* stock and the percent of shrunken flies in the F_2 determined. The wild strains from Belem and Iguassu gave significantly higher modification of the *2WB* norm than the other tested strains, producing respectively 29% and 14% shrunken flies. Values ranging from 5%–9% shrunken flies resulted from the crosses of the other wild strains to *2WB*.

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INCOMPATIBILITY IN AUTOTETRAPLOID WHITE CLOVER

II. Dominance and Double Reduction

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SELF- and cross-incompatibilities in *Trifolium repens* (white clover) are controlled by oppositional *S* alleles. The reaction of pollen is determined gametophytically, i.e., by the allele or alleles present in the pollen grain. Incompatibilities controlled by oppositional alleles of this type are dependent in autotetraploids, as they are in undoubled plants, on inhibitions of pollen germination and pollen-tube growth. Pollen of undoubled plants functions or fails depending on whether the *S* allele in the pollen is absent or present, respectively, in the style on which such pollen alights. In autotetraploids the diploid pollen may be either homogenic (e.g., S_1S_1) or heterogenic (e.g., S_1S_2) for *S* alleles. Homogenic pollen functions or fails in a manner similar to that of the corresponding haploid pollen. The reaction of heterogenic pollen is dependent on the interactions of the two different *S* alleles in the pollen. Two interactions designated competition and dominance have been reported in studies of autotetraploids of *Oenothera organensis* (Lewis 1947) and *Trifolium repens* (Atwood and Brewbaker 1953). Competition pollen classes functioned in styles of all genotypes in self- and cross-pollinations of 4N *T. repens* and 4N *T. hybridum* (Brewbaker 1954). Dominance pollen classes failed to effect fertilization when the dominant allele in the pollen was also present in the recipient style.

The following investigation was set up to study the dominance relationships in heterogenic pollen grains of four *S* alleles comprising two from each of two colchicine-doubled clones of white clover. The complication of competition interaction and self-compatibility (Brewbaker 1954) was obviated by selecting two self-incompatible (SI) parents of which all progeny were self-incompatible. Genotypic analyses of the F_1 and four F_1 backcross progenies selected for the elucidation of dominance patterns revealed the occurrence of double reduction resulting from crossing-over between the *S* locus and centromere in white clover.

MATERIALS AND METHODS

The origin of autotetraploid parental clones G4 and B1 of Ladino white clover has been described previously (Brewbaker 1954). Cultural methods and pollination techniques were the same as those reported. All flowering heads were trimmed to rings of ten florets which were suction-emasculated prior to pollination, and extreme caution was exercised to exclude contamination. Two rings of ten florets were self-pollinated on each of the two 4N parents and the 178 F_1 and backcross plants. All plants were classed as self-incompatible, and a general plant average of 0.3 selfed seed per ten florets was obtained. Selfed seed sets will not be given in the presentation of the analyses in this paper.

TABLE 1

Genotypes and groups expected in the progeny of G4 x B1 ($S_5S_5S_6S_6$ x $S_7S_7S_8S_8$) on the assumptions of chromosome and chromatid assortment ratios

Female gametes	Male gametes		
	1 (3)* S_7S_7	4 (8) S_7S_8	1 (3) S_8S_8
1 (3) S_5S_5	1 (9) $S_5S_5S_7S_7$ II_1	4 (24) $S_5S_5S_7S_8$ III_1	1 (9) $S_5S_5S_8S_8$ II_3
4 (8) S_5S_6	4 (24) $S_5S_6S_7S_7$ III_4	16 (64) $S_5S_6S_7S_8$ IV	4 (24) $S_5S_6S_8S_8$ III_3
1 (3) S_6S_6	1 (9) $S_6S_6S_7S_7$ II_4	4 (24) $S_6S_6S_7S_8$ III_2	1 (9) $S_6S_6S_8S_8$ II_2

* Figures in parentheses are calculated on the basis of random chromatid assortment ratios.

F_1 FAMILY

Colchicine-doubled parents G4 and B1 were crossed reciprocally, and 39 F_1 plants were studied. The theoretical expectation of this cross, assuming that the parents differ in both *S* factors, is the segregation in F_1 of nine *S* genotypes (table 1). Among the nine genotypes expected are four diallelics (II), four triallelics (III), and one tetra-allelic (IV). In order to distinguish the genotypes most readily, backcrosses of the F_1 plants as females to their parents were made first and were followed by F_1 intercrosses where necessary. These seed sets have been compiled in table 2, in which the F_1 plants are grouped into their respective genotypes as determined in the following series of steps:

(1) All F_1 plants were backcrossed as females to parental clones G4 and B1 as males. Three F_1 plants were cross-compatible with both parents, 15 were compatible with one of the parents and incompatible with the other, and 21 were incompatible with both parents (table 2). The compatibility relationships indicated that these groups were the diallelic (II), triallelic (III), and tetra-allelic (IV) com-

TABLE 2

Average seeds obtained from backcrosses of 39 F_1 plants to their SI 4N parents G4 and B1 ($S_5S_5S_6S_6$ and $S_7S_7S_8S_8$), and from selected intercrosses of F_1 plants

(In this and all subsequent tables, all seed set averages shown in **bold face type** are considered incompatible)

Group and genotype as female	Number of plants	Average seeds per 10 florets from crosses with plants of following genotypic groups as males								
		G4	B1	II_1	II_2	III_1	III_2	III_3	III_4	IV
G4-$S_5S_5S_6S_6$	1	—	13.0	20.0	5.0	8.7	12.4	13.0	19.0	18.0
B1-$S_7S_7S_8S_8$	1	6.9	—	15.0	14.0	—	6.0	9.0	6.6	—
II_1-$S_5S_5S_7S_7$	1	3.0	9.0	—	7.5	0.7	2.0	8.0	—	4.5
II_2-$S_6S_6S_8S_8$	2	9.3	17.0	18.5	**0.0**	14.8	7.5	3.0	8.5	7.5
III_1-$S_5S_5S_7S_8$	4	6.5	**0.4**	**0.0**	3.0	**0.0**	7.7	3.0	7.5	2.5
III_2-$S_6S_6S_7S_8$	3	10.2	**0.0**	11.0	**0.0**	10.2	**0.0**	1.5	3.6	4.2
III_3-$S_5S_6S_8S_8$	4	**0.1**	7.2	6.0	**0.5**	4.7	7.3	**0.3**	6.2	4.0
III_4-$S_5S_6S_7S_7$	4	**0.4**	8.8	**0.0**	24.0	1.0	5.8	13.0	**0.0**	10.6
IV-$S_5S_6S_7S_8$	21	**0.3**	**0.2**	**0.0**	**2.2**	**0.2**	**0.0**	**0 0**	**0.0**	**0.0**

binations, respectively, which were expected in a ratio of 1:4:4 on the basis of chromosome assortment ratios from colchicine-doubled parents having no *S* alleles in common (table 1).

(2) Intercrosses of the three diallelic F_1 plants (table 2) revealed two intra-sterile, inter-fertile groups, II_1 and II_2, with one and two plants, respectively.

(3) Seven of the triallelic F_1 plants were cross-compatible as females to G4 and cross-incompatible to B1. Intercrosses among these plants revealed two intra-sterile, inter-fertile groups, III_1 and III_2, with four and three plants, respectively.

(4) Eight of the triallelic F_1 plants were cross-incompatible as females to G4 and cross-compatible to B1. Intercrosses among these plants revealed two intra-sterile, inter-fertile groups, III_3 and III_4, each with four plants.

(5) One representative clone was selected from each of the seven F_1 groups. From two to four crosses were made for each of the possible combinations of the seven F_1 plants and their two parents. These data are summarized with those obtained from the four preceding steps in table 2.

The colchicine-doubled diallelic parents G4 and B1, thus shown to differ in both *S* alleles, were designated $S_5S_5S_6S_6$ and $S_7S_7S_8S_8$, respectively. The single plant of genotypic group II_1 was arbitrarily designated $S_5S_5S_7S_7$. The results summarized in table 2 were used to assign symbolic designations to the remaining F_1 groups. These were, respectively; II_2 ($S_6S_6S_8S_8$), III_1 ($S_5S_5S_7S_8$), III_2 ($S_6S_6S_7S_8$), III_3 ($S_5S_6S_8S_8$), III_4 ($S_5S_6S_7S_7$), and IV ($S_5S_6S_7S_8$). Of the nine genotypes which were expected (table 1) the two diallelic genotypes $S_5S_5S_8S_8$ and $S_6S_6S_7S_7$ were not obtained.

The seed set averages of crosses made for each of the 42 intercross combinations of the seven F_1 genotypes (table 2) were studied in an attempt to establish a tentative dominance series of the four *S* alleles. Gametic outlays were calculated on the basis of chromosome assortment ratios, i.e., assuming complete linkage of *S* locus and centromere. Certain of the 42 combinations, e.g., $S_5S_5S_7S_7$ x $S_5S_6S_7S_8$ were expected to be compatible due to the functioning of uninhibited gametes (S_6S_8 for the example given), while others, e.g., $S_5S_6S_7S_8$ x $S_5S_5S_7S_7$, were expected to set no seed due to the inhibition of all male gamete classes. Sixteen of the 42 combinations were of particular interest since they involved male parents which had a single allele not present in the female parent, e.g., $S_6S_6S_7S_8$ x $S_5S_6S_7S_8$. For each of these crosses, compatibility would indicate that one or more of the heterogenic male gametes bearing an allele also present in the female parent had effected fertilization as a result of S allele dominance in the pollen. At least two and as many as six of the 16 combinations involving single-allele differences were expected to be incompatible because of dominance on the assumption of chromosome assortment gametic outlays. However, all of the 16 combinations were partially or fully compatible, producing more seed than incompatible crosses or selfs (av. 0.3 seed). No dominance series could be devised to justify the apparent compatibility of all of these combinations.

It became evident upon examination of the theory that a weak link in the chain of reasoning (viz., ATWOOD and BREWBAKER 1953) was the assumption of complete linkage of *S* locus and centromere. Accordingly, the assumption was made that the *S* locus in white clover is located at some distance from the centromere, permitting

the occurrence of crossing-over and, upon the quadrivalent association of *S* locus chromosomes, of double reduction. Double reduction homogenic gametes were postulated to have effected fertilization in those crosses involving single-allele differences which were expected to be incompatible because of dominance on a chromosome basis. As an example, a tetra-allelic plant ($S_5S_6S_7S_8$) could produce pollen grains of the genotypes S_5S_5, S_6S_6, S_7S_7, and S_8S_8 by double reduction, whereas such homogenic gametes would not be produced if *S* locus and centromere were completely linked. All crosses involving single-allele differences, e.g., $S_6S_6S_7S_8$ x $S_5S_6S_7S_8$, would thus be expected to set a few seed upon fertilization by uninhibited homogenic gametes occurring as the result of double reduction (e.g., $S_6S_6S_7S_8$ fertilized by S_5S_5 double reduction gametes from $S_5S_6S_7S_8$).

In order to elucidate the dominance relationships and the postulated role of double reduction gametes, the following backcross progenies were studied.

BACKCROSS PROGENIES

A total of 139 plants were genotypically classified for *S* alleles in the four backcross (BC) progenies:

BC 1—$S_5S_5S_6S_6$ x $S_5S_5S_7S_8$ (G4 x III_1)
BC 2—$S_5S_5S_6S_6$ x $S_6S_6S_7S_8$ (G4 x III_2)
BC 3—$S_7S_7S_8S_8$ x $S_5S_6S_8S_8$ (B1 x III_3)
BC 4—$S_7S_7S_8S_8$ x $S_5S_6S_7S_7$ (B1 x III_4)

Insofar as possible, each of the plants in the four backcross progenies was selfed and testcrossed as female to parental clones G4 and B1 and to clones representing each of the seven F_1 genotypes. The seed sets of these crosses of BC plants have been compiled in table 3 with those from the F_1 inter- and back-crosses.

All BC plants were self-incompatible (av. 0.3 seed per 10 florets) and cross-incompatible within their respective genotypic groups (av. 0.3 seed). Each of the BC plants could be readily classified into its respective genotypic group on the basis of its compatibility as female to the four diallelics—G4 ($S_5S_5S_6S_6$), II_1 ($S_6S_6S_7S_7$), II_2 ($S_6S_6S_8S_8$), and B1 ($S_7S_7S_8S_8$). As in the F_1 progeny, diallelic BC plants were cross-compatible as females with three or all of the four diallelics as males, triallelics were cross-compatible with two of the four testers, and tetra-allelics were cross-compatible with none. All of the 11 genotypic groups (six diallelics, four triallelics, and one tetra-allelic) involving the four alleles were obtained. Each of the diallelic and triallelic genotypic groups comprised three genotypes, e.g. $S_5S_5S_6S_7$, $S_5S_6S_6S_7$, and $S_5S_6S_7S_7$ of group III_4, which could not be distinguished on the basis of the crosses made.

Before proceeding to interpret the individual backcross progeny segregations, it was necessary to establish the dominance relationships of the four *S* alleles. This was done by considering, in particular, the compatibility relationships of crosses involving single-allele differences among the summarized seed sets of the 1561 crosses made in the F_1 and four backcross progenies (table 3). In view of the postulated role of double reduction gametes, only those between-group crosses averaging fewer than 1.0 seed were considered to be incompatible (shown in bold face type in table

TABLE 3

Number of crosses and average seeds obtained when 39 F_1 plants of G4 x B1 and 139 BC plants were testcrossed

Group	Representative genotype	Number of plants	(Number of crosses) Average seeds per 10 florets from crosses with clones of following genotypic groups as males*								
			G4	B1	II_1	II_2	III_1	III_2	III_3	III_4	IV
G4	$S_5S_5S_6S_6$	1	—	(4)	(1)	(1)	(7)	(7)	(1)	(1)	(2)
				13.0	20.0	5.0	8.7	12.4	13.0	19.0	18.0
B1	$S_7S_7S_8S_8$	2	(6)	(1)	(2)	(3)	(2)	(2)	(17)	(13)	(2)
			7.8	**0.0**	12.0	9.0	2.0	5.0	9.0	6.0	2.0
II_1	$S_5S_5S_7S_7$	2	(3)	(3)	(2)	(5)	(4)	(3)	(5)	(3)	(4)
			3.7	9.0	**0.0**	9.0	**0.5**	11.3	7.6	4.0	9.8
II_2	$S_6S_6S_8S_8$	3	(5)	(5)	(5)	(8)	(9)	(6)	(7)	(7)	(5)
			11.2	11.8	17.0	**0.0**	13.0	9.0	3.9	8.7	6.4
II_3	$S_5S_5S_8S_8$	5	(5)	(5)	(5)	(6)	(6)	(5)	(3)	(6)	(6)
			9.2	7.8	8.4	7.3	3.3	10.6	11.3	11.2	7.3
II_4	$S_6S_6S_7S_7$	3	(3)	(3)	(3)	(5)	(3)	(3)	(2)	(2)	(2)
			5.0	8.0	10.0	4.6	7.0	10.3	3.0	12.0	3.5
III_1	$S_5S_5S_7S_8$	14	(16)	(14)	(10)	(16)	(17)	(16)	(10)	(12)	(15)
			4.7	**0.2**	**0.3**	4.2	**0.1**	7.2	2.7	2.7	1.8
III_2	$S_6S_6S_7S_8$	10	(15)	(13)	(11)	(18)	(16)	(10)	(9)	(20)	(14)
			8.2	**0.2**	5.5	**0.6**	7.8	**0.9**	3.0	2.6	4.4
III_3	$S_5S_6S_8S_8$	17	(24)	(22)	(19)	(23)	(19)	(17)	(10)	(27)	(16)
			0.1	7.3	8.6	**0.3**	2.4	5.7	**0.3**	5.7	1.7
III_4	$S_5S_6S_7S_7$	22	(28)	(25)	(20)	(31)	(22)	(23)	(19)	(31)	(24)
			0.5	7.3	**0.2**	10.9	**0.9**	3.6	7.0	**0.2**	7.0
IV	$S_5S_6S_7S_8$	66	(92)	(93)	(70)	(92)	(70)	(69)	(48)	(78)	(65)
			0.3	**0.3**	**0.3**	**0.7**	**0.3**	**0.2**	**0.2**	**0.2**	**0.5**

* Figures in parentheses = number of crosses.

3). The following steps were taken in the definition of the six dominance relationships of the four *S* alleles.

(1) Only two of the crosses involving single-allele differences were classed as incompatible. These were II_1 x III_1 ($S_5S_5S_7S_7$ x $S_5S_5S_7S_8$) and III_4 x III_1 ($S_5S_6S_7S_7$ x $S_5S_5S_7S_8$), which averaged 0.5 and 0.9 seed per cross, respectively (table 3). In each of these combinations the S_5S_5 and S_7S_7 gametes were expected to be inhibited, while heterogenic gamete classes S_5S_8 and S_7S_8 could theoretically have effected fertilization only if S_8 were dominant to alleles S_5 and S_7. The incompatibility of these two combinations (26 crosses) suggested that S_8 was recessive to both S_5 and S_7, i.e., that S_5 and S_7 were dominant to S_8.

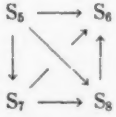
(2) The seed obtained in three compatible crosses which involved single allele differences—III_4 x III_2 ($S_5S_6S_7S_7$ x $S_6S_6S_7S_8$), III_4 x IV ($S_5S_6S_7S_7$ x $S_5S_6S_7S_8$), and II_4 x III_2 ($S_6S_6S_7S_7$ x $S_6S_6S_7S_8$)—could not have resulted from the functioning of S_7S_8 or of S_5S_8 pollen, since S_8 was adjudged in step (1) above to be recessive in each of these combinations. With the exception of S_6S_8 gametes, all other gamete classes were expected to be inhibited in these crosses. Therefore, it was deduced that the fertility of these three combinations (50 crosses, av. 5.6 seed) resulted from the functioning of S_6S_8 pollen, with S_8 dominant to S_6.

(3) The seed from two compatible crosses—III_1 x III_3 ($S_5S_6S_7S_8$ x $S_5S_6S_8S_8$) and II_3 x III_3 ($S_5S_5S_8S_8$ x $S_5S_6S_8S_8$)—could not have resulted from the functioning of S_6S_8 pollen according to step (2) above. The S_8S_8 and S_5S_8 pollen classes were expected to be incompatible. Consequently the fertility of these crosses was considered to have resulted from the functioning of S_5S_6 pollen, with S_6 dominant to S_5.

(4) The seed from two compatible crosses—III_2 x III_4 ($S_6S_6S_7S_8$ x $S_5S_6S_7S_7$) and II_4 x III_4 ($S_6S_6S_7S_7$ x $S_5S_6S_7S_7$)—could not have resulted from the functioning of S_5S_6 pollen according to step (3) above. The S_6S_7 and S_7S_7 pollen classes were expected to be incompatible. Consequently the fertility of these crosses was attributed to the functioning of S_5S_7 pollen, with S_5 dominant to S_7.

(5) The dominance relationship of alleles S_6 and S_7 was based on the interpretation of six of the compatible group intercrosses which involved single-allele differences. For the three combinations ($S_5S_6S_8S_8$ x $S_6S_6S_7S_8$, $S_5S_6S_8S_8$ x $S_5S_6S_7S_8$, and $S_6S_6S_8S_8$ x $S_6S_6S_7S_8$) in which S_7S_8 functioned because of dominance of S_7 over S_8, and in which S_6S_7 would be expected to function if S_7 were dominant to S_6, the 39 crosses averaged 4.6 seed per cross. In contrast, crosses in which S_7S_8 gametes functioned alone because of dominance (cf. table 13) averaged only 2.6 seed per cross. For the three combinations ($S_5S_5S_7S_8$ x $S_5S_6S_7S_7$, $S_5S_5S_7S_8$ x $S_5S_6S_7S_8$, and $S_5S_5S_7S_7$ x $S_5S_6S_7S_7$) in which S_5S_6 functioned because of dominance of S_6 over S_5, and in which S_6S_7 would be expected to function if S_6 were dominant to S_7, the 27 crosses averaged only 2.4 seed per cross. In comparison those crosses in which S_5S_6 gametes functioned alone because of dominance (table 13) averaged 3.1 seed per cross. It is suggested that S_6S_7 pollen functioned in the first three combinations in style bearing the S_6 allele, and failed in the latter three in styles bearing the S_7 allele, with S_7 exhibiting dominance over S_6.

The following non-linear pattern represents the dominance inter-relationships of the four alleles as defined:



No linear arrangement of the four alleles could be devised to explain the results satisfactorily. Linearity was evidenced in both of the previous studies of dominance relationships of *S* alleles in tetraploids (Lewis 1947, Atwood and Brewbaker 1953).

The interpretation of individual backcross segregations necessarily followed the establishment of the dominance relationships of the four S alleles. The consideration of observed BC segregations which follows was made on the basis of HALDANE's limiting chromatid assortment gametic ratios.

BC 1, G4 x III_1 ($S_5S_5S_6S_6$ *x* $S_5S_5S_7S_8$)

The 34 plants of BC 1 segregated into six genotypic groups, in the ratio of 1 II_3:5 III_1:2 III_2:5 III_3:2 III_4:19 IV (table 4). Applying HALDANE's formulae, the gametic segregation of male parent III_1 ($S_5S_5S_7S_8$) would have been 6 S_5S_5:8 S_5S_7:8

TABLE 4

Average seeds obtained when 34 plants of BC 1, G4 x III_1 ($S_5S_5S_6S_6$ x $S_5S_5S_7S_8$), were testcrossed to clones of 9 related and 1 unrelated genotypes

Group and genotype	Number of plants	Average seeds per 10 florets from crosses with plants of following genotypic groups as males									Average seeds to unrelated tester
		G4	B1	II_1	II_2	III_1	III_2	III_3	III_4	IV	
II_3-$S_5S_5S_8S_8$	1	2.0	3.0	7.0	4.5	0.0	0.0	4.0	6.0	1.0	7.0
III_1-$S_5S_5S_7S_8$	5	3.2	**0.0**	**0.2**	4.6	**0.2**	6.2	3.2	2.0	1.0	17.5
III_2-$S_6S_6S_7S_8$	2	8.0	**0.5**	2.5	**0.5**	3.0	**2.0**	2.0	0.0	1.5	24.0
III_3-$S_5S_6S_8S_8$	5	**0.0**	6.0	8.3	**0.0**	1.8	5.0	**1.0**	4.7	1.2	15.0
III_4-$S_5S_6S_7S_7$	2	**1.0**	6.0	**1.0**	11.0	**0.5**	10.5	3.0	**1.7**	0.0	30.0
IV-$S_5S_6S_7S_8$	19	**0.4**	**0.7**	**0.7**	**0.6**	**0.3**	**0.4**	**0.1**	**0.2**	**0.6**	13.2

TABLE 5

Genotypes expected in BC 1, G4 x III_1 ($S_5S_5S_6S_6$ x $S_5S_5S_7S_8$) on the basis of random chromatid assortment ratios

Female gametes	Male gametes								
	6 S_5S_5 (0)*	8 S_5S_7 (0^D)	8 S_5S_8 (0^D)	4 S_7S_8 (+)		1 S_7S_7 (+)		1 S_8S_8 (+)	
3 S_5S_5	—	—	—	12 $S_5S_5S_7S_8$	III_1	3 $S_5S_5S_7S_7$	II_1	3 $S_5S_5S_8S_8$	II_3
8 S_5S_6	—	—	—	32 $S_5S_6S_7S_8$	IV	8 $S_5S_6S_7S_7$	III_4	8 $S_5S_6S_8S_8$	III_3
3 S_6S_6	—	—	—	12 $S_6S_6S_7S_8$	III_2	3 $S_6S_6S_7S_7$	II_4	3 $S_6S_6S_8S_8$	II_2

* (+) means that pollen should function
(0) means that pollen should not function
($+^D$) means that pollen functions because of dominance interaction
(0^D) means that pollen does not function because of dominance, i.e., it would function if dominance relations were reversed.

S_5S_8:4 S_7S_8:1 S_7S_7:1 S_8S_8 (table 5). The S_5S_5 homogenic pollen grains were inhibited (0), and the S_5S_7 and S_5S_8 heterogenic were considered to be inhibited by dominance (O^D), with S_5 exhibiting dominance over both alleles S_7 and S_8. The 26 plants in groups III_1, III_2, and IV could be attributed to the functioning of the uninhibited (+) S_7S_8 pollen grains (table 5). The eight remaining plants (2 III_4, 5 III_3, and 1 II_3) are believed to have resulted from the functioning of S_7S_7 and S_8S_8 double reduction pollen grains.

BC 2, G4 x III_2 ($S_5S_5S_6S_6$ x $S_6S_6S_7S_8$)

The 15 plants of BC 2 segregated into five genotypic groups in the ratio of 2 II_2:1 III_1:6 III_3:3 III_4:3 IV (table 6). Applying HALDANE's formulae, the gametic segregation of male parent III_2 ($S_6S_6S_7S_8$) would have been 6 S_6S_6:8 S_6S_7:8 S_6S_8:4 S_7S_8:1 S_7S_7:1 S_8S_8 (table 7). The S_6S_6 homogenic pollen grains were inhibited (0). The four plants in groups III_1 and IV could be attributed to the functioning of the uninhibited (+) S_7S_8 pollen grains. The three plants of group III_4 could have resulted from fertilization by S_6S_7 dominance or by S_7S_7 double reduction gametes. Similarly,

TABLE 6

Average seeds obtained when 15 plants of BC 2, G4 x III$_2$ ($S_5S_5S_6S_6$ x $S_5S_6S_7S_8$), were testcrossed to clones of 9 related genotypes

Group and genotype(s)	Number of plants	Average seeds per 10 florets from crosses with plants of following genotypic groups as males								
		G4	B1	II$_1$	II$_2$	III$_1$	III$_2$	III$_3$	III$_4$	IV
II$_2$-$S_6S_6S_6S_8$, or $S_6S_6S_8S_8$	2	14.0	7.5	27.5	**0.0**	9.0	14.0	9.0	7.0	3.5
III$_1$-$S_5S_5S_7S_8$	1	6.0	—	**1.0**	2.5	**0.0**	20.0	—	—	0.0
III$_3$-$S_5S_5S_6S_8$, or $S_5S_6S_6S_8$, or $S_5S_6S_8S_8$	6	**0.3**	5.2	8.0	**0.4**	2.0	5.4	—	3.2	0.8
III$_4$-$S_5S_5S_6S_7$, or $S_5S_6S_6S_7$, or $S_5S_6S_7S_7$	3	**0.0**	6.0	**0.0**	11.3	**0.0**	6.0	—	—	—
IV-$S_5S_6S_7S_8$	3	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**	**0.0**	**0.0**	**0.0**	**0.0**

TABLE 7

Genotypes expected in BC 2, G4 x III$_2$ ($S_5S_5S_6S_6$ x $S_6S_6S_7S_8$) on the basis of random chromatid assortment ratios

Female gametes	Male gametes					
	6 S_6S_6 (0)	8 S_6S_7 (+^{D})	8 S_6S_8 (+^{D})	4 S_7S_8 (+)	1 S_7S_7 (+)	1 S_8S_8 (+)
3 S_5S_5	—	24 $S_5S_5S_6S_7$ III$_4$	24 $S_5S_5S_6S_8$ III$_3$	12 $S_5S_5S_7S_8$ III$_1$	3 $S_5S_5S_7S_7$ II$_1$	3 $S_5S_5S_8S_8$ II$_3$
8 S_5S_6	—	64 $S_5S_6S_6S_7$ III$_4$	64 $S_5S_6S_6S_8$ III$_3$	32 $S_5S_6S_7S_8$ IV	8 $S_5S_6S_7S_7$ III$_4$	8 $S_5S_6S_8S_8$ III$_3$
3 S_6S_6	—	24 $S_6S_6S_6S_7$ II$_4$	24 $S_6S_6S_6S_8$ II$_2$	12 $S_6S_6S_7S_8$ III$_2$	3 $S_6S_6S_7S_7$ II$_4$	3 $S_6S_6S_8S_8$ II$_2$

the 2 II$_2$ and 6 III$_3$ plants could have resulted from fertilization by either the S_6S_8 dominance gametes (+^{D}) or by uninhibited (+) S_8S_8 double reduction gametes.

BC 3, B1 x III$_3$ ($S_7S_7S_8S_8$ x $S_5S_6S_8S_8$)

The 51 plants of BC 3 segregated into eight genotypic groups in the ratio of 2 II$_1$:1 II$_2$:4 II$_3$:3 III$_1$:4 III$_2$:3 III$_3$:9 III$_4$:25 IV (table 8). Applying HALDANE's formulae, the gametic segregation of male parent III$_3$ ($S_5S_6S_8S_8$) would have been 1 S_5S_5:1 S_6S_6:4 S_5S_6:8 S_5S_8:8 S_6S_8:6 S_8S_8 (table 9). The S_8S_8 homogenic pollen grains were inhibited, and the S_6S_8 heterogenic pollen grains were inhibited by dominance of S_8 over S_6. The 37 plants in groups III$_3$, III$_4$, and IV could be attributed

TABLE 8

Average seeds obtained when 51 plants of BC 3, B1 x III_3 ($S_7S_7S_8S_8$ x $S_5S_6S_8S_8$), were testcrossed to clones of nine related genotypes

Group and genotype(s)	Number of plants	Average seeds per 10 florets from crosses with plants of following genotypic groups as males								
		G4	B1	II_1	II_2	III_1	III_2	III_3	III_4	IV
II_1-$S_5S_6S_7S_7$	2	4.0	9.0	**0.0**	10.0	**0.0**	16.0	6.0	4.0	15.0
II_2-$S_6S_6S_8S_8$	1	14.0	10.0	3.0	**0.0**	14.0	10.0	3.0	13.0	10.0
II_3-$S_5S_5S_8S_8$, or $S_5S_8S_8S_8$	4	11.0	9.0	8.8	8.8	5.0	13.2	15.0	12.2	8.6
III_1-$S_5S_6S_7S_8$, or $S_5S_7S_7S_8$, or $S_5S_7S_8S_8$	3	3.0	**0.0**	**0.3**	4.2	**0.0**	5.7	0.0	1.5	2.5
III_2-$S_6S_6S_7S_8$	4	8.2	**0.5**	6.8	**0.6**	7.2	**1.0**	4.3	1.8	6.0
III_3-$S_5S_6S_8S_8$	3	**0.0**	11.0	8.0	**1.0**	2.3	6.0	**0.0**	7.0	0.5
III_4-$S_5S_6S_7S_7$	9	**0.4**	7.9	**0.2**	14.6	**1.8**	2.7	2.4	**0.1**	8.0
IV-$S_5S_6S_7S_8$	25	**0.1**	**0.3**	**0.2**	**0.7**	**0.4**	**0.2**	**0.5**	**0.2**	**0.9**

TABLE 9

Genotypes expected in BC 3, B1 x III_3 ($S_7S_7S_8S_8$ x $S_5S_6S_8S_8$) on the basis of random chromatid assortment ratios

Female gametes	Male gametes			
	1 S_5S_5 (+)	1 S_6S_6 (+)	4 S_5S_6 (+)	8 S_5S_8 ($+^D$)	8 S_6S_8 (0^D)	6 S_8S_8 (0)
3 S_7S_7	3 $S_5S_5S_7S_7$ II_1	3 $S_6S_6S_7S_7$ II_4	12 $S_5S_6S_7S_7$ III_4	24 $S_5S_7S_7S_8$ III_1	—	—
8 S_7S_8	8 $S_5S_5S_7S_8$ III_1	8 $S_6S_6S_7S_8$ III_2	32 $S_5S_6S_7S_8$ IV	64 $S_5S_7S_8S_8$ III_1	—	—
3 S_8S_8	3 $S_5S_5S_8S_8$ II_3	3 $S_6S_6S_8S_8$ II_2	12 $S_5S_6S_8S_8$ III_3	24 $S_5S_8S_8S_8$ II_3	—	—

to functioning of the uninhibited S_6S_6 pollen grains (table 9). Five plants (1 II_2, 4 III_2) are believed to have resulted from fertilization by S_6S_6 double reduction gametes, and two plants (II_1) from fertilization by S_5S_5 double reduction gametes. The two plants in genotypic group II_1, having only alleles S_5 and S_7, could be accounted for in no other way than by the fertilization of S_7S_7 female gametes by S_5S_5 double reduction gametes from the male parent $S_5S_6S_8S_8$. The remaining seven plants (4 II_3, 3 III_1) could have resulted from the functioning of either the S_5S_8 dominance pollen grains or of S_5S_5 double reduction grains.

BC 4, B1 x III_4 ($S_7S_7S_8S_8$ x $S_5S_6S_7S_7$)

TABLE 10

Average seeds obtained when 39 plants of BC 4, B1 x III$_4$ ($S_7S_7S_8S_8$ x $S_5S_6S_7S_7$), were testcrossed to clones of nine related and one unrelated genotypes

Group and genotype(s)	Number of plants	Average seeds per 10 florets from crosses with plants of following genotypic groups as males									Average seeds to unrelated tester
		G4	B1	II$_1$	II$_2$	III$_1$	III$_2$	III$_3$	III$_4$	IV	
B1$_{BC}$-$S_7S_7S_7S_8$, or $S_7S_7S_8S_8$, or $S_7S_8S_8S_8$	1	12.0	**0.0**	9.0	6.5	4.0	4.0	—	2.5	2.0	28.0
II$_4$-$S_6S_6S_7S_7$	3	5.0	8.0	10.0	4.6	7.0	10.3	3.0	12.0	3.5	18.5
III$_1$-$S_5S_5S_7S_8$, or $S_5S_7S_7S_8$, or $S_5S_7S_8S_8$	1	5.0	**0.0**	**0.0**	7.0	**0.0**	2.0	2.0	1.0	2.0	13.0
III$_2$-$S_6S_6S_7S_8$	4	5.8	**0.0**	4.5	**0.8**	7.7	**1.0**	3.5	2.6	4.2	19.8
III$_3$-$S_5S_6S_8S_8$	3	**0.0**	10.0	11.7	**0.2**	2.3	6.0	—	7.8	3.2	6.0
III$_4$-$S_6S_6S_7S_7$	8	**0.8**	6.7	**0.0**	7.3	**0.3**	0.3	0.2	**0.0**	3.5	17.8
IV-$S_5S_6S_7S_8$	19	**0.2**	**0.1**	**0.1**	**0.7**	**0.2**	**0.0**	**0.2**	**0.1**	**0.2**	11.2

TABLE 11

Genotypes expected in BC 4, B1 x III$_4$ ($S_7S_7S_8S_8$ x $S_5S_6S_7S_7$) on the basis of random chromatid assortment ratios

Female gametes	Male gametes													
	1 S_5S_5 (+)		1 S_6S_6 (+)		4 S_5S_6 (+)		8 S_5S_7 (+^{D})		8 S_5S_7 (0^D)	6 S_7S_7 (0)
3 S_7S_7	3 $S_5S_5S_7S_7$	II$_1$	3 $S_6S_6S_7S_7$	II$_4$	12 $S_5S_6S_7S_7$	III$_4$	24 $S_5S_7S_7S_7$	II$_1$	—	—
8 S_7S_8	8 $S_5S_5S_7S_8$	III$_1$	8 $S_6S_6S_7S_8$	III$_2$	32 $S_5S_6S_7S_8$	IV	64 $S_5S_7S_7S_8$	III$_1$	—	—
3 S_8S_8	3 $S_5S_5S_8S_8$	II$_3$	3 $S_6S_6S_8S_8$	II$_2$	12 $S_5S_6S_8S_8$	III$_3$	24 $S_5S_7S_8S_8$	III$_1$	—	—

The 39 plants of BC 4 segregated into seven genotypic groups in the ratio of 1 B1$_{BC}$:3 II$_4$:1 III$_1$:4 III$_2$:3 III$_3$:8 III$_4$:19 IV (table 10). Applying HALDANE's formulae the gametic segregation of the male parent III$_4$ ($S_5S_6S_7S_7$) would have been 1 S_5S_5:1 S_6S_6:4 S_5S_6:8 S_5S_7:8 S_6S_7:6 S_7S_7 (table 11). The S_7S_7 pollen grains were inhibited, and the S_6S_7 pollen grains were inhibited by dominance of S_7 over S_6. The 30 plants in groups III$_3$, III$_4$, and IV resulted from the functioning of the uninhibited S_5S_6 pollen grains. One plant (B1$_{BC}$) was cross-incompatible only with its female parent, and apparently resulted from fortuitous self-fertilization or cross-fertilization by a S_7S_7 homogenic gamete of the male parent. The one plant of group III$_1$ could be ascribed to S_5S_7 heterogenic dominance gametes or to S_5S_5 double

reduction gametes. Seven plants (3 II_4 and 4 III_2) were attributed to fertilization by the S_6S_6 double reduction gametes.

DISCUSSION

Linkage of S locus and centromere

The postulate that *S* locus and centromere in white clover are separated at a sufficient distance to permit crossing-over and the occurrence of double reduction was set up as a basis for the interpretation of the data presented. ATWOOD and BREWBAKER (1953) recognized the likelihood that *S* locus and centromere were not completely linked, although they found no convincing evidence for the occurrence of double reduction. In the present study, 22 backcross plants belonged to genotypic classes which were expected upon the fertilization by double reduction gametes. The frequency with which double reduction gametes functioned in the four backcrosses has been utilized in the following discussion as the basis for the calculation of map distance between *S* locus and centromere.

Double reduction frequencies can be directly applied in MATHER's formulae (MATHER 1936) for the calculation of the map distance of gene to centromere, assuming a given degree of quadrivalent association. In the four white clover backcrosses the homogenic double reduction gametes could be expected to function with a frequency similar to that of other uninhibited gametes. Thus the relative rates of fertilization by double reduction homogenic and uninhibited heterogenic gametes are assumed to have reflected directly the frequencies with which these types were produced, in turn reflecting the frequency of crossing-over between *S* locus and centromere.

It was not possible to definitely assign any of the 15 plants in BC 2 to double reduction classes, and the results obtained in this family are not considered in the following calculations. Of the 124 plants in the three remaining backcrosses (BC 1, 3, and 4) 22 were believed to have resulted from fertilization by double reduction gametes. A total of 93 plants in these three progenies were attributed to the functioning of the uninhibited heterogenic pollen classes. One plant in BC 4 was classed as a fortuitous self. Seven plants in BC 3 and one in BC 4 could have resulted from fertilization by either the homogenic double reduction or the heterogenic dominance gametes. For the calculations that follow, these eight plants were attributed to the dominance gametes. The ratios of plants resulting from fertilization by double reduction homogenic gametes to plants resulting from fertilization by uninhibited heterogenic gametes in the pertinent families were (a) BC 1, 8:26, (b) BC 3, 7:37, and (c) BC 4, 7:30 (total, 22:93). For each of these families the random chromatid assortment ratio for these classes is 1:2. Applying MATHER's formulae (MATHER 1936) this calculated ratio of double reduction to uninhibited classes is $\alpha:\frac{2-\alpha}{3}$, or 3:5 when alpha is assumed to be the maximum $\frac{1}{3}$ (i.e., when it is assumed that all *S* locus chromosomes associate in quadrivalents, with maximum crossing-over between *S* locus and centromere).

MATHER's alpha equals the product of e = frequency of equational separation (a function of gene to centromere map distance) and a = frequency of genetic non-

disjunction (a function of the degree of quadrivalent association). If it is assumed that $a = \frac{1}{3}$, i.e., that all S locus chromosomes associated as quadrivalents, the ratio $\alpha:\frac{2-\alpha}{3}$ becomes $e:\frac{6-e}{3}$. The latter ratio may be equated with the observed ratio of 22:93, and e calculated to be 43.95. In other words the S locus is placed at 43.95/2 or approximately 22 units from the centromere by this calculation. If S locus chromosomes associated in quadrivalents less than 100 per cent of the time, the map distance is underestimated by this value. In addition, any or all of the eight plants of BC 3 and BC 4 which were arbitrarily assigned to dominance gametes could have been attributed to the functioning of homogenic double reduction gametes. The calculated map distance of 22 units is thus considered only as a minimum value.

In several instances segregating data of ATWOOD and BREWBAKER (1953) and of the current study were found to give more satisfactory Chi-square values when fitting to chromosome than to chromatid assortment ratios. In each of these cases the segregations also conform satisfactorily to ratios based on the calculated 22 units map distance. An illustration of these calculations is provided by a consideration of the gametic outlays of parental clones G4 and B1. The gametic segregation of G4 as female is found to be 10 S_5S_5:48 S_5S_6:6 S_6S_6, and that of B1 as female is 23 S_7S_7:59 S_7S_8:10 S_8S_8. The respective totals of 49 homogenic and 107 heterogenic gametes were used for the following calculations. The expected chromosome assortment ratio of gametes from a diallelic tetraploid is 1:2, which satisfactorily fits the observed segregation (Chi-square = 0.130, P = .80–.70); the expected chromatid assortment ratio is 6:8, from which the observed segregation is statistically discrepant (Chi-square = 7.886, P < .01). The expected gametic outlay, applying MATHER's formulae, is $1 + \alpha$ homogenic:$2 - \alpha$ heterogenic. Utilizing the calculated $e = 43.95$, alpha equals 0.1462. Substituting this value into the ratio of $1 + \alpha$:$2 - \alpha$, the calculated gametic outlay of diallelic tetraploid parents used in the present study is 2.29 homogenic:3.71 heterogenic. The observed ratio of 49 homogenic:107 heterogenic is satisfactorily fitted by this calculated ratio (Chi-square = 2.771, P = .10–.05).

Dominance and functional disadvantage

Nineteen of the 139 backcross plants were classified as resulting from fertilization by heterogenic gametes which functioned because of dominance. Any of these plants may have resulted instead from fertilization by double reduction gametes. If all 19 plants are attributed to dominance gametes, the ratios of plants resulting from fertilization by dominance ($+^D$) gametes to plants resulting from fertilization by uninhibited heterogenic (+) gametes in the three families for which such data was available are

(1) BC 2—observed 11 $+^D$:4 +, expected ratio 4:1,

(2) BC 3—observed 7 $+^D$:37 +, expected ratio 2:1, and

(3) BC 4—observed 1 $+^D$:30 +, expected ratio 2:1. The expected ratios are the same regardless of assumptions concerning linkage of S locus and centromere. While the observed ratio in BC 2 was similar to that expected, each of the other two families showed a significant discrepance from expectation. In each of the latter

TABLE 12

Seed sets and calculated percents of functional pollen of selected crosses in table 3

Types of functional pollen	Percent functional pollen*	Number of crosses	Average seed per cross
Dominance heterogenic	16.7	92	4.0
Dominance heterogenic	33.3	64	3.8
Uninhibited heterogenic	16.7	129	7.6
Uninhibited heterogenic	100.0	68	13.9

* Calculated on the basis of chromosome assortment gametic ratios.

families, pollen grains which functioned because of dominance effected fertilization much less frequently than expected on the assumption of the equality of functional abilities of uninhibited and dominance gametes. A similar discrepancy was observed by ATWOOD and BREWBAKER (1953), who reported that pollen grains which functioned because of dominance were often partially inhibited, suggesting that *S* allele dominance was incomplete for most combinations.

The partial inhibition of dominance gametes may be denoted clearly in the seed sets of compatible crosses summarized in table 3. Pertinent seed set data are summarized in table 12, together with the corresponding percentages of pollen capable of effecting fertilization. The percentages of functional pollen were calculated on the basis of chromosome assortment ratios for convenience in interpretation. These values differ only slightly from those expected on the basis of the map distance of 22 units calculated in the preceding section.

The 68 crosses in which all of the pollen was uninhibited averaged 13.9 seed per cross (table 12). The 129 crosses in which a single uninhibited pollen class, comprising $\frac{1}{6}$ of the pollen grains on a chromosome basis, functioned alone to effect fertilization set an average of 7.6 seed per cross. Crosses in which single heterogenic dominance gametes (plus the corresponding uninhibited double reduction gametes) effected fertilization comprised two groups with 17 and 33 per cents functional pollen, respectively. The average seed sets of these crosses were 4.0 and 3.8, respectively, or about one half that obtained from comparable crosses involving uninhibited gametes.

The data in table 12 from the 156 crosses for which seed was attributed to dominance gametes are presented in another way in table 13. Individual dominance

TABLE 13

Seed sets of crosses (table 3) in which seed resulted from the functioning of single heterogenic dominance classes

Heterogenic pollen class	Dominance relationship	Number of crosses	Average seed per cross*
S_5S_6	$S_6 > S_5$	43	3.1
S_5S_7	$S_5 > S_7$	22	3.5
S_5S_8	$S_5 > S_8$	16	3.3
S_6S_8	$S_8 > S_6$	50	5.6
S_7S_8	$S_7 > S_8$	25	2.6

* LSD at the 5 percent level equals 1.0.

classes were distinguished, and the seed sets attributable to each are given. The 50 crosses in which S_6S_8 gametes functioned alone set considerably more seed than the other classes. A significant component of the variance among the five means (MS = 55.651, F = 3.071, P = .05–.01) existed in the comparison, for one degree of freedom, of the S_6S_8 average with the mean of the other four averages (F = 11.77, P < .01). This can be interpreted to mean that the various dominance combinations differed in degree of dominance, with the relationship of $S_8 > S_6$ being that of a relatively strong dominance as compared with the others. The genotypic segregation of BC 2 considered above lends support to this conclusion. ATWOOD and BREWBAKER (1953) observed similar differences in degree of dominance.

The occurrence of dominance among *S* alleles in heterogenic pollen grains appears to be supported by a large body of evidence from this and previous studies (cf., LEWIS 1947, ATWOOD and BREWBAKER 1953). However, the evidence from the present investigation is not regarded as establishing conclusively the six dominance relationships. This is due in large part to the role of double reduction gametes in the crosses made. The possibility exists that certain *S* alleles show complete independence of action in heterogenic pollen grains, in which case such heterogenic combinations would be inhibited on styles bearing either or both of the alleles in the pollen. "Independence" has been recognized by several investigations dealing with oppositional *R* alleles of the sporophytic type, in which dominance is manifest at the diploid level (cf. GERSTEL 1950, HUGHES and BABCOCK 1950).

Relationship of seed set and percent functional pollen

The relationship of seed set to percent of functional pollen in compatible crosses of 4N white clover which was described by BREWBAKER and ATWOOD (1952) and ATWOOD and BREWBAKER (1953) is illustrated in table 12. Crosses in which all of the pollen was potentially able to effect fertilization (including B2 testcrosses in BC 1 and BC 4) set nearly twice as many seeds (av. 13.9 seed per cross) as those crosses in which only 1/6 of the pollen, on a chromosome assortment basis, was uninhibited (av. 7.6 seed per cross). Corresponding values from ATWOOD and BREWBAKER (1953) are (a) 100 percent functional pollen—av. 24.8 seed per cross; (b) 17 percent functional pollen—11.0 seed per cross.

TABLE 14

Seed sets (table 3) grouped according to percents of functional pollen

Percent functional pollen[1]		Number of crosses	Average seed per cross*
Average	Range		
0.0	—	935	0.3
3.4	2–6	211	4.1
20.6	17–25	362	7.7
53.1	50–56	38	9.8
100.0	—	68	13.9

* LSD of compatible crosses at the 5 percent level equals 2.7.

[1] Calculated on a chromosome assortment basis.

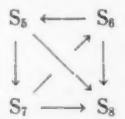
ATWOOD and BREWBAKER (1953) calculated that, of the five dominance gametes of their investigation, at least three functioned approximately 1/8 as frequently as uninhibited gametes. Applying this value for "functional disadvantage" to their data, an equation of $Y = .19\ X + 8.72$ was calculated to express the regression of seed yield on percent functional pollen. The value of 1/8 functional disadvantage was similarly applied to pollen grains which functioned because of dominance in the 99 genotypic combinations in table 3, and theoretical percents of functional pollen were calculated for all combinations. Chromosome assortment gametic ratios were applied for convenience in calculation. The 1609 data were grouped into five classes of percents of functional pollen ranging from incompatible to fully compatible (table 14). The average seed sets of the four compatible groups ranged from 4.1 to 13.9 and the corresponding average percents of functional pollen ranged from 3.4 to 100. These values were correlated with $r = .947$. The regression equation for these data was $Y = .16X + 1.91$, which differs from that cited above largely in the point of origin of the regression line. This may be accounted for by the comparatively low seed sets of Ladino white clover clones used in the present investigation.

SUMMARY

1. Two self-incompatible (SI), colchicine-doubled diallelic clones of white clover were crossed, and 39 F_1 and 139 backcross (BC) plants were genotypically classified for oppositional *S* alleles.

2. The two 4N parents were shown to differ in both *S* alleles, and were designated $S_5S_5S_6S_6$ and $S_7S_7S_8S_8$, respectively. All 11 genotypic groups representing the 6 diallelic, 4 triallelic, and 1 tetra-allelic combinations of the four *S* alleles were obtained. All plants were self-incompatible and cross-incompatible within their respective genotypic groups.

3. Dominance relationships of the four alleles in the six different heterogenic pollen classes were determined on the basis of the seed sets of 1561 crosses of F_1 and BC plants. The following non-linear dominance series was established:



4. Pollen grains which functioned because of dominance were much less effective than uninhibited grains in effecting fertilization, as reflected in both seed sets and progeny segregations. In other words, dominance grains showed a functional disadvantage in comparison to uninhibited grains, indicating weak or partial dominance of all combinations. The dominance of S_8 over S_6 appeared to be significantly stronger than others with which it could be statistically compared.

5. Crossing-over between the *S* locus and centromere in white clover was evidenced by the segregation and function of double reduction homogenic *S* gametes. The map distance from *S* locus to centromere was estimated to be 22 units on the assumption of 100 percent quadrivalent association of *S* locus chromosomes.

6. Seed sets (Y) were correlated with the percents of pollen (X) which were theo-

retically functional in compatible crosses. The calculated regression equation for this relationship was $Y = .16X + 1.91$.

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